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PROGRESS IN AUTOPHARMACOLOGY[1]

A Survey of Present Knowledge of the Chemical Regulation of Certain Functions by Natural Constituents of the Tissues

SIR HENRY H. DALE, M.A., M.D., F.R.C.P., F.R.S.

I. INTRODUCTION. THE ACTION OF HISTAMINE, AND THE EVIDENCE RESTRICTING ITS PROBABLE SIGNIFICANCE. EVIDENCE FOR OTHER NATURAL VASODILATORS

The invitation to lecture here on the Dohme Foundation has naturally carried my mind back, with a grateful recognition of the double honour which the University has thus bestowed upon me, to my first visit to this country, now nearly 14 years ago, to deliver the Herter Lectures (1). On that occasion the observations and the problems, which formed the subject of my lectures, were given some kind of ordered connection by their relation to a central idea, which was that of finding a chemical basis for a complex of reactions common to a number of physiological and pathological phenomena. These reactions were largely, though by no means exclusively, concerned with changes in the efficiency of the circulation due to variations in tone of the smallest blood-vessels, including the capillary vessels, of which the independent activity, and its importance for efficient circulation, had then come newly into prominence, especially in the analysis of conditions termed circulatory shock. During the then recent war several investigators had independently been led by their observations to the idea of a chemical cause of the circulatory failure, which formed a central feature in some of the conditions which had been called wound shock, and in other shock-like conditions, such as that of the acute anaphylactic reaction. Though many details of the work of that period have needed revision, in the light of investigations made under the unhampered and unhurried conditions of more normal times,

[1] Delivered under the Charles E. Dohme Memorial Lectureship, Johns Hopkins University School of Medicine, April 20, 21 and 22, 1933.

it brought into new prominence the factors requisite for the maintenance of the efficient circulation, as the central condition of a normal bodily activity, and it aroused new interest in the part which might be played by chemical factors in more finely regulating the circulation, or in determining its collapse. Up to that period, exact knowledge of chemical factors, which might be concerned in this vital function, had been practically limited to the discovery, many years earlier, of the hormones, predominantly augmentor in their effects on vascular tone—epinephrine from the suprarenal medulla, and the pressor principle of the pituitary posterior lobe. For the rest, it was known that a simple extract from almost any organ or tissue would show evidence of the presence of some substance or substances having a generally depressant effect on vascular tone, usually spoken of as "depressor substances," or, on the theory that one widely distributed principle was concerned in the common action of so many extracts, as "vasodilatin." In the same way the largely similar symptoms of the anaphylactic reaction had been attributed to the new production of a hypothetical "anaphylatoxin." A hint that pure chemistry might eventually play its part in unravelling these phenomena had been given by the chance discovery by my co-workers and myself, some 10 years earlier, that the substance histamine, obtained from an entirely different natural source, produced a number of the symptoms common to the effects of tissue extracts and to the anaphylactic reaction. Interest in the action of histamine had been revived and strengthened by the analogies found between some of its effects, when subjected to a closer analysis, and some of the symptoms seen in certain types of wound shock. My colleagues and I had endeavoured to stand firm against the obvious temptation to assume a direct relationship; we had consciously avoided the suggestion that, whenever symptoms appeared resembling those produced by the injection of histamine, that substance itself was concerned. We knew at that time very little concerning the presence of histamine as a natural constituent of the tissues. Barger and I (2), indeed, had many years earlier isolated and identified traces of it from one animal tissue, but under conditions which seemed to us to leave the cause of its occurrence there open to doubt. And shortly before my earlier visit here, Professor Abel (3) had again obtained it, under conditions less open to criticism than ours, indeed, but from

materials and by methods which had seemed to me still to leave us without complete certainty that histamine was a normal constituent or product of the living animal tissues. And even if we could assume it to be so, we had then no data to justify any theory as to the conditions of its production or liberation in life, or as to the mode of its intervention in normal or morbid bodily reactions. The position has undergone a great and progressive change in the interval. I think we can say now that histamine is a natural constituent of the living cells of most organs and tissues of the body. And I think that we now have the data for defining much more clearly the probable rôle of this naturally occurring histamine.

The recognition of histamine as a natural body constituent, and the gradual acquisition from many sources of evidence indicating and delimiting its probable function, have played some part, I think, in opening a wider line of enquiry. The course and the manner of the quest have varied. You will remember that histamine was known as a chemical substance, and that its intense activity, so suggestively resembling some effects of tissue extracts, had been discovered before there was any suspicion of its occurrence in the body. The activity had led us to look for it in the body, and to find it. The same has since occurred in at least one other case; the intense and characteristic physiological action of a substance first known as an artificial product of the laboratory has again given the clue to its discovery as a natural body constituent. In more than one other instance the course of discovery has been the converse of this; effects produced by a tissue extract have apparently given the clue to the identification in the tissues of a substance producing such effects; and this, in one case, has proved to be a substance, or group of substances, already known to occur there, but not previously known to have such a pharmacodynamic action. In other cases the clue of activity has led to the isolation of substances which, according to present evidence, appear to be new. In yet other cases, powerful principles have been separated and shown to possess activities of characteristic types, but their purification has, hitherto, stopped short of chemical isolation.

It will be clear, I think, that knowledge in this special field has advanced rapidly from the position of fourteen years ago. And though I have attempted twice in that interval to estimate the progress which

had been made to those intermediate dates, and to revise the interpretation of old and new evidence in the light of it, the recent additions to knowledge have been so numerous and varied, that the time seems ripe for a further survey of progress, and for a wider consideration of the changes of view which it involves. The purpose of the Dohme Lectureship is "to promote the development of a more intimate relationship between chemistry, pharmacy and medicine." If I use this opportunity to review the extent to which chemistry has now resolved some of the problems in this field of what I have ventured to call "autopharmacology," and the light which these discoveries throw on the natural control of functions by chemical means, I think that my subject will have a proper relation to the object of the Foundation; and its choice will provide a link between these lectures and those which I gave here on the Herter Foundation, which, I hope, may add to the interest of both.

Histamine

I must first pass in more detailed but still rapid review the steps in the very great change that has taken place in our knowledge of the direct significance of histamine and its action, since I last lectured here. I then assumed the rôle of the cautious sceptic. Histamine was known to reproduce in a striking manner certain features of a vascular reaction to irritation or injury of the tissues. Professor Abel had gone further than my colleagues and I in producing evidence for the presence of histamine in tissue extracts, and he was prepared to go further than I was, at that time, in suggesting a direct physiological rôle for this substance. The sequel has shown that Professor Abel's courage was more justified than my caution. I think it has been so on other occasions. It must be remembered, however, that we were then only considering a physiological intervention of histamine, in terms of its possible new production, by some decarboxylating decomposition of protein, occurring in response to stimulus or injury. The histamine which had been found in certain tissue extracts was regarded as probably the product of an immediate *post-mortem* change; I myself was not quite convinced that even the possibility of bacterial action had been rigidly excluded. On the other hand, the fact that the union of antigen and antibody, in the anaphylactically sensitive cell, pro-

duced a response so closely simulating that of a normal cell to histamine, which some would have interpreted as showing that histamine was formed as the result of that union, had suggested to me that we might be dealing with the direct responses of the various types of cells to aggregating changes in their protoplasm, and that histamine was merely one of many possible agents producing a physical change of this kind.

I think that we were all, and I am certain that I was at that time, overlooking by far the most probable explanation of these resemblances. Nobody, I think, then suspected that the normal cells of various tissues, apart from any injury or irritation, might contain, and somehow hold in their protoplasm, significant amounts of histamine, and that the effect of irritation or injury might be, not to cause histamine to be newly formed, but to destroy the conditions which normally kept it inside the cells, so that it would escape into the tissue fluids, and produce its intense physiological effects, especially on the neighbouring small blood vessels.

If this conception had been in the mind of any of those working in this field fourteen years ago, it could not have presented itself as much more than a plausible hypothesis. Even so it might have led us earlier to the experiments needed to test its validity. As it happened, the experimental evidence came first, and the theory arose from the necessity of explaining it. We needed evidence, firstly, that histamine occurs naturally in the living cells of various tissues, and that its presence in a tissue extract is not the result of *post-mortem* change. In the second place, evidence was needed that irritation or injury, even of a very mild type, causes the cells to shed, into the fluids bathing them, a substance having the action of histamine.

Taking first the chemical evidence, though this did not come first in order of time, the renewal of the effort to identify the tissue constituent acting like histamine owed its origin to somewhat accidental circumstances. An extract of fresh ox liver, made and purified by a procedure involving fractionation with alcohol and acetone, had been used clinically in Toronto for the treatment of high blood pressure, with initially promising results. Dr. C. H. Best, visiting my laboratory at the time, brought with him a supply of this extract, and directions for the preparation of more, with a view to a closer physiological

analysis of its properties, and an eventual attempt to isolate and identify its vasodilator constituent. The physiological analysis showed that all the vasodilator effects of this particular extract, together with its actions on other organs, were covered by actions of histamine and choline, or of the two in combination, with the resulting potentiation of their effects. The latter point was the subject only of incidental observation, but is worthy of passing notice. Our previous information had indicated that histamine alone, in a cat under ether, would produce a general dilator action on the minutest blood-vessels, accompanied by a relatively weak constrictor action on those somewhat larger. On these latter, on the other hand, choline had appeared to produce a powerfully dilator effect, with little if any direct influence on the tone of the capillaries. If we were right in thus locating their respective actions, these two bases acting together, and simultaneously opening all the vessels which could participate in the production of the peripheral resistance, should give a vasodilator depression exceeding the sum of those produced by the two substances when acting singly—a true physiological potentiation. Best put this deduction to the test of experiment, and was able to demonstrate its correctness. This observation warned us of the complications to be expected in dealing with the action of a crude extract on quantitative lines; but it also encouraged sufficient confidence in our measurements to enable our chemical colleagues, Drs. Dudley and Thorpe, to use them as a guide, and not merely to demonstrate that histamine and choline were present, but to isolate them in sufficient quantities to account for the vasodilator actions of this extract (4). This did not mean, of course, that they would account for all the vasodilator effects of all liver extracts; still less for those of all extracts from all other organs. It did suggest, however, that, when not merely a vasodilator action, but one of the special histamine type, was found to be produced by an organ extract, the action was probably due to the presence of histamine itself. The question remained, how it came there. For the study of this we required an organ of which a simple alcoholic extract showed the histamine action in greater intensity, and greater physiological purity. We found this in the lung, the depressor effect of an extract from which closely resembled that of pure histamine, and readily yielded the base in sufficient quantity to account for practically the whole of its phar-

macodynamic activity. It was easy then to show that one lung, excised from the living animal and dropped directly into alcohol so cold that the tissue was immediately frozen, yielded as much histamine as the other lung, on which the extraction was carried out some hours after death. So far then as evidence can be obtained at all of the pre-existence of a substance in living cells, on evidence as good as that which tells us that lactic acid, for example, exists already in the living muscle, we could state that the living, uninjured cells of the lung contain histamine, in an amount sufficient to produce an even violent physiological reaction, if it should by any means be suddenly liberated into the circulation (70 mgm. per kilo). And in the case of other tissues which have been examined, we have the same warrant for believing that the smaller proportions of histamine which they yield are present in them during life, and are not formed by autolytic or other changes after death.

Meanwhile an imposing body of evidence, showing that the local vascular reaction to irritation or injury is evoked by a chemical stimulus, had been accumulating through the work of Sir Thomas Lewis and his co-workers. These studies have been assembled by Lewis (5) in his book on "The blood-vessels of the human skin and their reactions," published in 1927. Early in their course, Lewis had become convinced that the three-fold reaction of the skin vessels following all kinds of local irritation or injury—localised capillary dilatation, oedema similarly localised, and a reflex arteriodilator flush in the surrounding area—could only be explained by the appearance in the immediate neighbourhood of the affected epidermal cells of a substance causing all these effects. Some years before, Sollmann and Pilcher (6) had demonstrated that histamine, among other substances, produced capillary dilatation and whealing when introduced into the human epidermis. When, therefore, Sir Thomas Lewis asked me to suggest a known substance which could be tried in such a connection, I handed him histamine, but with no real expectation of the extraordinary series of resemblances which he and his co-workers were to trace, between the effects of all types of injury, and those of histamine in high dilutions introduced by light puncture into the epidermis. By the time our own chemical studies had reached the point just mentioned, those of Lewis and his colleagues were ready to meet them. Harris (7) had shown

that extracts of epidermis were second only to those from lung in their potency in producing the three-fold vascular skin-response in man, as in producing a vasodilator fall of blood-pressure in the anaesthetized cat under atropine, a test commonly used by us in following histamine through a series of chemical separations. Since histamine itself had not, and indeed has not yet been chemically separated from skin extracts, Lewis cautiously referring to their active constituent as "H-substance," the question outstanding was whether the H-substance, which appeared when the living epidermal cells were injured, was newly formed by some chemical change which the injury initiated, or was simply liberated from cells which previously held it, inert in their interior. Our own evidence on the natural occurrence of histamine had reached the point at which it told strongly in favour of the latter view; and this was directly confirmed by an experiment of Harris, in which he showed that scalding produced no immediate change in the amount of H-substance which could be extracted from a given area of living skin, but that an equal area, marked out beforehand and then scalded, gave a diminished yield as compared with an equal normal area, as soon as it had become definitely oedematous, so that fluid containing the released H-substance was draining from it along the lymphatic channels.

We seemed, therefore, to have at last a clear interpretation of those striking similarities between the complex of symptoms produced by histamine and those occurring in various types of physiological and pathological reactions. Histamine was present preformed in the protoplasm of the cells of a large number of the organs and tissues of the body, normally held there in some loose combination, or by a specific impermeability of the normal cell membrane, but easily released by any influence upsetting these normal conditions of its retention. The chemical experience obtained in the isolation of histamine, showing the readiness with which it formed molecular complexes with other constituents of a tissue extract, so that special methods had to be devised for its release from such associations, made it easy to accept the idea of its retention in the normal cell, and its liberation in response to disturbance of any kind. As to the precise mechanism of that retention, however, we can still make no more definite suggestion than we can, for example, as to the conditions which enable a resting cell, such as a

muscle fibre, to hold potassium ions in its interior, and to resist their interchange with the sodium ions of the surrounding medium. The reproduction of the effects of histamine in the symptoms of the anaphylactic shock, with their differences in different species, found now not merely a plausible, but a satisfactory explanation. There was now no need to suppose that the union of antigen and antibody caused the formation of histamine by some newly initiated fermentative action—an idea which I had found so difficult, when I last spoke here, to reconcile with the almost immediate reaction of the sensitized cell to the antigen. The histamine was ready in the interior of the cell, and the union of the antigen with the precipitating antibody, in the cell protoplasm, had only to provide the physical disturbance required for its release. This conception of the anaphylactic reaction, moreover, could be put to the test of practical experiment. Manwaring, who first showed that the liver cells of the dog form the seat of the primary reaction in the anaphylactic shock of that species (8), was able to show that the blood leaving the liver during the shock, conveyed by a cross-circulation scheme into the vessels of another and normal dog, would elicit in other organs of the latter, such as the bladder, a reaction similar to that produced by histamine (9). Gebauer-Fülnegg, Dragstedt and Müllenix (10) have confirmed these observations, and shown that the substance can be detected in the lymph leaving the dog's liver during the anaphylactic shock, and that it shows no properties which are incompatible with its being histamine. Another elegant demonstration is that given by Bartosch, Feldberg and Nagel (11), using the isolated lung of an anaphylactic guinea-pig perfused with Ringer's solution, under which conditions, as I observed many years ago, it responds to the sensitizing antigen with the typical bronchial constriction. Catching the solution flowing from the veins of such a lung, these authors were able to show that, during the reaction produced by adding the sensitizing protein to the inflowing solution, a substance leaves the lungs which will produce constriction of the bronchioles in the similarly perfused lungs of a normal guinea-pig, and which, in all other directions in which it has been tested, produces actions corresponding to those of histamine, including, as a negative example, its failure to stimulate the muscle of the rat's uterus. It is either histamine itself, or something physiologically indistinguishable from it.

Bartosch, Feldberg and Nagel (12) were further able to test directly whether the histamine-like principle in the effluent was produced by some cleavage of the antigen, according to the older view, or was liberated from the cells of the lung. They found that it reached its maximum concentration after practically the whole of the antigen had passed out of the system, and concluded that it must come from the lung cells, in which our own experiments had shown it to occur in such an exceptionally large proportion.

This view of the significance of histamine in the body, and of the mode of its intervention to produce natural vascular and other reactions, provided such a satisfactory explanation for the resemblances which had puzzled us, as to create a new temptation to undue haste in generalizing its significance. Whenever a reaction of a vasodilator type was seen, without any nervous mechanism to account for it, it was as easy now to say "histamine" as it used to be to say "metabolites," to rest content with an easy assumption or, perhaps, to spend time in finding plausible explanations for facts which the liberation of histaine would still not explain without subsidiary assumptions. It will be clear, however, that new knowledge was more likely to come from a study of those effects of tissue extracts, and those natural vascular reactions, which histamine would not reproduce, than from a search for more examples of the now familiar resemblances. From the outset of our acquaintance with its action, my colleagues and I had drawn attention to the fact that, though histamine would produce the central symptoms of the anaphylactic shock with remarkable fidelity, there were characteristic features of the shock which were missing in its action. A good example was the loss of the coagulability of the blood in the dog. When we realize, however, that the primary event in the shock, in this species, is a direct injury of the liver cells, it is clear that histamine, though the dramatic intensity of its action may give a predominance to its effects in the ensuing syndrome, may be only one of the constituents liberated from the liver cells subjected to such injury; and the production of something which destroys the natural coagulability of the blood is a common reaction of the liver cells of the fasting dog to various poisons, and not merely of the anaphylactic cells to the specific antigen. The discrepancy reminds us, however, that, though we had good reason to suppose that histamine

was released from cells of certain types when they were injured or irritated, and that in such cases it might account for the principal features of the local vascular reaction which ensued, we had no right to assume that it was, in any case, the only substance so released, or that from all tissues and in all species it was even the principal agent in vascular reactions to injury. It was clear that all assumptions as to its probable function, based on insufficiently critical evidence, needed careful review, and that the phenomena which it would *not* reproduce deserved a more direct attention. I propose to consider some of these cases, in which a more careful study has shown that histamine cannot be responsible for effects which loose deduction from analogy might have disposed us to attribute to it.

I have mentioned already the analogy traced, during the war period, between the shock-like effects produced in the cat and the dog by large doses of histamine, and the symptoms recorded in certain types of wound shock. At about the same time Professor Cannon and the late Sir William Bayliss, among others, had come to the conclusion, based partly on clinical data but largely on an experimental study of the effects of massive trauma, that the circulatory failure, in secondary wound shock, was due to absorption of toxic products from damaged tissues, and especially from damaged muscles. I do not think that the suggestion that histamine was the product in question was ever explicitly made; but our description of the action of histamine was much under discussion as providing an analogy, and had, indeed, played a part in turning attention to the possibility of a toxaemia as a cause of shock. As a result, I think that an impression was abroad that histamine itself, or something very much like it, was released from damaged muscle, to act as the cause of shock. More recent evidence, acquired under more normal conditions, has failed to substantiate such an idea. In the first place, when we had methods for isolating histamine from the tissues, and came to make a survey of the various organs for its presence, the voluntary muscles, although small quantities were eventually isolated from them with great difficulty by Dr. Thorpe (13), were found to contain a much smaller proportion of histamine than any of the other tissues which we examined. Evidence to be discussed later has shown that, if vasodilator substances from voluntary muscle were still to be considered as potential factors in the

production of shock, our attention would have to be directed rather to adenosine and its derivatives. The whole theory of a toxaemic origin of shock following muscle trauma, however, has meanwhile been under experimental review. M. I. Smith (14), of Washington, in a very careful series of experiments, failed to find any evidence of absorption of shock-producing substances from crushed leg muscles, though histamine, artificially introduced into the damaged limb, showed its effects on the general circulation readily, when the limb vessels were released. Smith concluded that the really important factor, in the shock-like effect following such experimental trauma, was the effusion with stagnation of blood in the wounded tissues, and not the absorption of toxic products from them. Simonart (15), who has also called in question the interpretation which Bayliss and Cannon gave to their results, attributes them, on the other hand, to nervous effects on the vasomotor centres, thus returning to an older conception. Whichever interpretation is right, it is clear that the newer experimental evidence is unfavourable to the general conception of toxaemia as the principal cause of the experimental shock produced by muscle trauma; and if we accept that fundamental revision, the question whether histamine is likely to be the toxic agent no longer arises in that case.

On the other hand, I think we should be unwise too hastily to give a general application to these negative conclusions, from a particular tissue and form of experiment. There is excellent evidence that something acting exactly like histamine can escape into the general circulation and produce its typical effects at a distance, in response even to a very mild trauma of tissues like the epidermis, which are rich in that base. Lewis (*loc. cit.*) found that the production of wheals over an extensive area of skin, in a patient liable to factitious urticaria, would produce a flushing of the cheeks, the intensity of which could be matched with that produced by a subcutaneous dose of histamine. Kalk (16), in a similar patient, found that the production of wheals on the skin of the legs evoked a secretion of acid gastric juice—another typical histamine effect. We should be unwise, I think, on the ground of negative results of experiments on the crushing of the histamine-poor voluntary muscles, to discount altogether the possibility that irritative handling and exposure of abdominal viscera, for example, which are relatively rich in it, might liberate histamine into the circulation

in shock-producing amounts. It is extremely difficult to get direct evidence on such a point, and no positive theory could be justified without it; on the other hand, a negative theory, excluding such a possibility on imperfect analogy, would have even less justification at present.

From a very early period in our knowledge of the action of histamine, there have been other and simpler reasons for hesitation in attributing too general a significance to it. It was early shown that its most characteristic action on the circulation, *viz.* the production of a vasodilator fall of arterial pressure by very small doses, was not seen in all animals. It occurs in the carnivora, in the monkey and, as we now know, in man, though with some difference of detail in its incidence, which seems to be limited to the ultimate arterioles and capillaries in the cat, but to involve somewhat larger arterioles also in the dog and in man. On the other hand, it could not be seen in the rodents, in which, under the ordinary conditions of anaesthesia, it appeared to produce nothing but an arterial constriction, causing a moderate rise of arterial pressure. More recent observations made by Feldberg (17) during a visit to my laboratory, have shown that in a rabbit anaesthetized with chloralose histamine will show a moderate depressor, vasodilator effect, and that in the denervated ear of the rabbit, without anaesthesia, an injection of histamine into the vein of the other ear will cause visible arterial constriction, accompanied by an obvious, capillaridilator flush. Even in the rabbit, therefore, histamine has a relaxing effect on capillary tone, though the effect of this is masked, in an ordinary blood-pressure record, by that of a concomitant constriction of the arteries.

But though, on such evidence, we could still allow to histamine a possible function of some significance in the natural vascular reactions even of the rabbit, evidence of a different kind made it clear that there were other substances still to be considered. In some of the earliest observations published on the depressor effects of tissue extracts, Swale Vincent and his co-workers (18) had shown that some of these would cause the arterial pressure to fall in anaesthetized rabbits. The effect was not due to choline, since it persisted after atropine had been given. When the action of histamine was discovered, it soon became clear that it would no more explain this item in the depressor

effects of tissue extracts than choline would. The detailed analysis of the action of histamine, and its identification as a natural tissue constituent, preoccupied for a time the interest of many workers in this field. There was, however, this other aspect of the total activity still to be accounted for, and the hope that eventually another substance, or other substances, would be chemically identified, with actions of a potency comparable to that of histamine, and perhaps as significant in the interpretation of natural vascular reactions, especially in certain species.

The failure of histamine to produce in the frog's circulation its characteristic vasodilator depression, as seen in the carnivora, was another of the earlier observations of Laidlaw and myself. The work of Grant and Jones (19), in Sir Thomas Lewis's department, made quite clear the failure of histamine to reproduce the natural vascular reactions to injury in this species. They found that an extract of frog's skin, on the other hand, when lightly pricked into the surface of the frog's tongue, would produce a vascular reaction as closely simulating the response to local injury of the mucosa as the effects of histamine would simulate the injury response of vessels in the human skin. On the other hand, this substance in the frog's skin, to which its own vessels were sensitive, was relatively ineffective on the vessels of the skin of man.

The rat and mouse furnish the most striking examples, among mammals yet investigated, of insensitiveness to the action of histamine, of which relatively enormous doses must be injected to produce any significant symptoms in these animals. This might well have been regarded as explaining the well-known difficulty of producing anaphylactic sensitiveness in the muridae, and the weakness of the reaction when it occurs. Professor Longcope (20), however, provided a sufficient explanation apart from this, in showing that the precipitating antibody of a rat will not sensitize the tissues, even of such a sensitive anaphylactic reagent as the guinea-pig. Kellaway (21) found, on the other hand, that rabbit precipitin for a foreign protein, such as egg-albumin, would passively sensitize the plain muscle of a rat's uterus, so that it now responded to egg-albumin by contraction. No explanation of the anaphylactic reaction by a release of histamine from the cells will meet this case. For the muscle of the rat's uterus is not

merely very insensitive to histamine; it responds by relaxation to doses of that base which are large enough to affect it at all. We must either interpret the contractile anaphylactic response of this organ, in any case a relatively weak one, as a direct response to the antigen-antibody union in its fibres, or suppose that some substance other than histamine is released, which stimulates them to contraction. We have only considerations of general probability to guide us. I do not think that we could make a strong case for this feeble and artificial anaphylactic response being certainly due to a mechanism analogous to that concerned in the intense reactions of other species. On more general grounds, however, it does not seem likely that the rat and the mouse would altogether lack such a chemical mechanism of protective vascular reaction, to injury or irritation of the tissues, as histamine provides in the species in which such reactions have been more closely studied. It seems more likely that the reaction detected by Kellaway is due to the release of a substance which performs in the muridae the function subserved by histamine in the primates and the carnivora, and which, in accordance with that function, stimulates the muscle fibres of the rat's uterus to contraction.

When we look at this more recent evidence as a whole, it does not appear to weaken the case for histamine as a chemical stimulant concerned in normal vascular and other bodily reactions. On the contrary, it seems to strengthen it, while giving it precision and defining more clearly the limits of its validity. As the lines of that part of a general picture which the actions of histamine cover were more firmly drawn, the blank spaces, waiting to be filled by a similarly detailed study of the actions of other substances, came more clearly into view. The possibility that a significant part in vascular reactions might be played by choline, much longer known than histamine as a natural body constituent, seemed to need a more careful consideration. Then there were vasodilator substances, certainly different from histamine and choline, still to be identified in many tissue extracts. There were also reactions which, from experience of species sensitive to it, we might refer to the "histamine type," occurring in species not giving such reactions with histamine. In the work-a-day phraseology current for some years in my laboratory, we needed knowledge yet of the frog-histamine, the rat-histamine, possibly the rabbit-histamine; one

substance might fill these various rôles, or several might yet be needed. In later lectures I shall consider which of these gaps have now been filled, and with what success. It may then, I hope, be possible to obtain a better idea of the whole picture, at the present stage of its development. Meanwhile, I hope we can leave, for the present, that part of it concerned with histamine, with the feeling that it has gained in precision and coherence.

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II. ACETYLCHOLINE: ITS NATURAL OCCURRENCE AND PROBABLE FUNCTION. ADENOSINE AND ITS DERIVATIVES

In my last lecture I referred to the desirability of a new and more careful consideration of the possibility that choline, long known as a constituent of many organs, might also play a part in evoking local vascular reactions. The exact description by Lewis and his co-workers of the reactions of the skin vessels did not support the idea of such a

direct participation; for the reaction in immediate proximity to an effective stimulus was found by them to be restricted to the minute vessels—the capillaries and the smallest arterioles and venules adjoining them; whereas the action of choline would certainly be expected to involve larger arterioles. It seemed possible, therefore, that the separation of choline from protoplasmic complexes might require some more deep-seated disturbance than that which sufficed for the release of histamine. For years, however, there had existed a suspicion that not choline itself, but its enormously more active acetic ester might play a part in natural body reactions; but this possibility hovered just below the important horizon which separates speculation from experimental fact. The remarkable activity of acetylcholine had been discovered in 1906 by Hunt and Taveau (1), who had observed in extracts from the suprarenal gland, and also from brain, a depressor activity, too intense to be accounted for by the choline which it contained, and apparently due to some derivative, which easily split off choline at any attempt to isolate it. Hunt was led to examine, with Taveau, a series of choline derivatives, and thus came across the intense activity of acetylcholine. Later it came directly to my own notice (2), as other interesting substances had done, through the accident of its occurrence in a particular ergot extract. I was led to examine its action more in detail, and to emphasize the remarkable fidelity with which it reproduced the effects of stimulating parasympathetic nerves—a resemblance comparable to that between the actions of epinephrine and the effects of true sympathetic nerves. The almost unique intensity, combined with the evanescence of its action, the latter, as I predicted, due to rapid hydrolysis by an esterase in the blood, led me to suggest that acetylcholine would be admirably fitted to play the part of a parasympathetic hormone, if only there were any reason to suppose that it actually occurred in the animal body. This was pure speculation at the time, and the facts which have substantially justified it came much later.

The first clear indication that acetylcholine might intervene in a natural reaction of the animal body came with the demonstration by Otto Loewi (3) and his co-workers, from 1920 onwards, that the effects of nerve-impulses in the vagus, in inhibiting the heart-beat of the frog, are transmitted by a chemical substance, which escapes during such

inhibition into the Ringer's solution filling the heart, in sufficient quantity to enable the inhibition to be transmitted to another heart by transfer of the solution. This fundamental observation was soon confirmed by Hamburger and his coworkers, among others, and Professor Hamburger (4) described his experiments fully in the first series of lectures delivered on the Dohme Foundation. Professor Loewi will be lecturing in this country in the near future, and I shall only draw your attention to such items, of this now classical demonstration of the chemical transmission of nerve impulses, as bear on the nature of the substance responsible. Every observation made by Loewi and his colleagues was compatible with its being acetylcholine. It was certainly a choline ester, easily hydrolysed, and thereby rendered practically inert, by the action of an esterase in the heart muscle; the activity being restored by artificial acetylation of the residue. The long known potentiating effect of physostigmine on the action of the vagus and other parasympathetic nerves was seen in a new light, when Loewi found that physostigmine inhibited the destructive action of the heart esterase on the vagus hormone. The later demonstration by Engelhart (5), in Loewi's laboratory, and by Matthes (6) in my own, that physostigmine similarly stops the action of an esterase in the mammalian blood, by which acetylcholine is normally destroyed with great rapidity, provided another striking parallel between the vagus substance and acetylcholine. Atropine did not prevent the liberation of the vagus substance, but prevented it from acting, just as it prevents the action of all choline esters.

All this circumstantial, indirect evidence was entirely favourable to the identification of the vagus substance as acetylcholine. It was certainly a choline ester of remarkably similar properties; but direct chemical evidence was long lacking, even as to the existence of acetylcholine, or of any other active choline ester, as a natural constituent of the body. Eventually this came, from an unexpected quarter. As an item in our survey of the tissues for histamine Dudley and I (7) had decided to examine the spleen, since extracts of this organ had been described as containing a principle which acted like histamine but, it was suggested, differed from it in chemical behaviour. We accordingly investigated extracts from the spleens of horses and oxen,

and had no difficulty in showing that the substance acting like histamine, as in other cases, was histamine itself. Two visiting investigators from this country, Drs. Walter Bauer and Dickinson Richards, who had been carrying out some of the physiological assays for us, drew my attention to the extraordinary potency of a choline-like action shown by these spleen extracts. This was easily eliminated by atropine, so that the histamine determinations were not affected: but a simple calculation showed that the activity which atropine thus suppressed would correspond to the presence in the extract of a quantity of choline amounting to 20 or 30 grammes per kilogramme of fresh spleen tissue. This in itself seemed extremely improbable; and when it appeared that the substance producing the choline-action readily disappeared if the solution was made alkaline, it became very probable that we were dealing with a naturally occurring choline ester, resembling acetylcholine in its intense activity and its instability. Following this clue, Dr. Dudley isolated the substance, obtaining it as pure double salts with the chlorides of platinum and gold, and proved it to be acetylcholine itself.

Acetylcholine, therefore, was a substance natural to the animal body; but it was difficult, and remains so, to attribute any definite physiological significance to its occurrence in the spleen, where we had found it. Its presence was difficult to associate with any function of the spleen itself. On the other hand, acetylcholine is destroyed with such extreme rapidity in the blood—of a small dose added to blood *in vitro* upwards of 90 *per cent.* has vanished in half a minute at room temperature—that it seemed little suited to play the part of a true hormone, and to be distributed in the blood, like epinephrine, so as to evoke simultaneously the effects of parasympathetic nerves on the various organs of the body. And the spleen would appear to be, on the whole, one of the least appropriate organs in the body to form and store it, and to release it for such a purpose, since any acetylcholine leaving the spleen would have to run the gauntlet of the liver and its activities, before it reached even the right side of the heart. Again, there is no evidence known to me of the physiological occurrence of a sudden, general parasympathetic stimulation, comparable to the general production of sympathetic effects by accelerated release of epinephrine in response to urgent emotions, for which Professor Cannon has presented the evidence.

Altogether, then, we found it difficult to account for this occurrence of physiologically important amounts of acetylcholine in the spleens of oxen and horses. And the difficulty was not diminished by our failure to find it in the spleens of other animals which we examined, including both carnivorous and herbivorous species. The most plausible suggestion which presented itself, to explain these stores in the spleens of the larger ungulates, was that the acetylcholine might somehow be carried from them by the blood in such a way that it would be prevented from manifesting its intense physiological activity, and be protected from destruction by the blood esterase. We thought, for example, that it might somehow be held inside the blood corpuscles. If we could suppose it to be so transported, and deposited at the periphery, still in an inactivating and protective complex, to await release at the call of parasympathetic nerve impulses, we should have at least a plausible conception of its appearance in the place where we found it, and of its ultimate contribution to known physiological effects.

I think I can say that such additional evidence as we have acquired from our own observations is, on the whole, compatible with such a conception. Such traces of acetylcholine as my colleagues and I have been able to detect in the blood, by physiological tests, appear to be in the corpuscles. We can only say, however, that there is a trace of activity corresponding to that of acetylcholine, and showing the characteristic intensification by physostigmine when tested on tissues such as frog's voluntary muscle (*rectus abdominis*) or the body wall of the leech. In other tissues, on the other hand, larger quantities, though still small in comparison with that in the spleen of the ox or horse, have been similarly detected by my colleague Dr. Gaddum with Professor Chang (8) of Peking; and the occurrence seemed to be, in general, related to the known distribution of parasympathetic nerves to plain muscle and gland cells.

A comparison was made of the relative activities of a series of choline esters on different types of isolated organ; and it was found that, of the esters tested, only the pyruvyl-ester showed activities on the different physiological reagents in the same proportion as those of acetylcholine, its actions being in each case weaker to the same extent. I may remind you that some years ago Le Heux, in Magnus's labora-

tory, testing the effects of a number of salts on the isolated intestine, came to the conclusion that pyruvates shared with the acetates a property of intensifying choline action, and attributed these effects to a natural formation of the corresponding esters in the living tissue. It may be said, then, that the choline ester found by my colleagues in different organs, and showing a general correspondence in its distribution to parasympathetic innervation, could only be acetylcholine or pyruvyl choline, of the esters hitherto tested. And since acetylcholine is, of these, the one known to occur in the body, and much the more active, the probability is strongly in its favour.

I must faithfully record, however, results of a widely different nature, with regard to the occurrence and distribution of acetylcholine in the body, which have been obtained in other laboratories. We were ready, as I have explained, to find evidence of the occurrence of this substance in the circulating blood, under conditions preventing both its activity and its destruction. Accordingly we read with special interest the account, by Professor Kapfhammer and his colleagues in Freiburg (9), of the isolation of substantial quantities—as much as 30 or 40 milligrammes per litre—of acetylcholine from fresh ox blood. We hastened to repeat this important observation; but I am bound to report that, in spite of many attempts, we have consistently failed to confirm it (9a). There is no possibility of doubt that the substance obtained by the Freiburg investigators was acetylcholine. We have been in repeated and friendly interchange of experience with them, and Dr. Dudley has visited Freiburg to obtain every exact detail of their method. Nevertheless, on applying it, we again regularly failed to find in blood, even when taken directly from the living animal, evidence of the presence of acetylcholine in more than such minimal traces as to preclude any hope of chemical separation. The Freiburg workers, on the other hand, have extended the range of their enquiries, and have obtained even larger quantities of acetylcholine from various organs and tissues (10). I may mention, in particular, their finding of nearly 200 milligrammes per kilogramme of fresh skeletal muscle. They also put the skinned carcasses of whole animals—dogs, cats and rabbits—through the mincing machine, and extracted about 50 milligrammes of acetylcholine per kilogramme of total body weight. I should mention further, the fact that Vogel-

fanger (11), in the laboratory of Professor Parnas at Lwow, has recently confirmed the Freiburg observations, by finding acetylcholine in ox blood in the same high proportion. Our own experience fails to confirm that of the Freiburg investigators with regard to any tissue except one—the human placenta. In this, as in the spleens of horses and oxen, we have detected acetylcholine in such quantities in the direct alcoholic extract, without further chemical treatment, that a simple physiological test cannot fail to recognize it. But for its presence there it is even more difficult to suggest a physiological function. In the rabbit's placenta Chang and Gaddum found no acetylcholine.

The whole question, as you will see, of the presence and distribution in the body of this intensely active substance, is at present the subject of a clear-cut discrepancy in the experience of different laboratories, without any present prospect of a reconciling explanation. We have considered, without success, all possibilities of the occurrence of acetylcholine as an accidental contamination in reagents, such as alcohol, which are used in large quantities in the extraction. We must leave the conflict of evidence as it stands for the present. Our own evidence, by itself, indicates the presence in certain organs, such as the spleen and the placenta of some species, of acetylcholine in quantities which seem to have no physiological meaning as yet. For the rest, we find it widely distributed in small amounts, and in a manner generally according with the supposition that it is concerned with the action of parasympathetic nerves. On the other hand, the Freiburg findings seem to have no relation to any physiological function which we can conceive. In the skeletal muscle, where the only known innervation of parasympathetic type is that of the vasodilator endings of sensory fibres on arterioles, they show the highest content of any tissue in the body. In the heart, again, their findings show large amounts of acetylcholine in the mammalian ventricle, with no direct vagus control, and relatively little in the auricle; in contrast with the findings of my own colleagues, whose results, within their much lower range, show a reverse relationship for the occurrence in auricle and ventricle, in accordance with physiological expectation from the distribution of parasympathetic nerves. One thing is quite clear, that acetylcholine, in the quantities found by the Freiburg laboratory in different tissues, cannot exist in the free condition, or in any form in

which it could readily be liberated. The quantity found by them in a single gramme of muscle, if suddenly introduced into the circulation of a cat, would produce an intense physiological reaction, including a prolonged and complete inhibition of the heart.

Leaving aside, then, this mysterious chemical discrepancy, with the confident hope that the future will resolve it, we may look a little closer at the physiological evidence for the intervention of acetylcholine in natural functions. Loewi's demonstration of the release of something having these properties, from the frog's heart under vagus stimulation, has been extended by Engelhart (12) to the effect of the third cranial nerve on the mammalian iris, and by Babkin (13) and by Gibbs (14) to the effect of the chorda tympani on the submaxillary gland. Babkin showed that, when one chorda tympani is stimulated, there passes into the blood something, which, if physostigmine is present to prevent its destruction, causes not only a fall of arterial pressure, but a secretion from the opposite salivary gland. Gibbs succeeded in catching this substance in the Ringer's solution flowing from a perfused salivary gland during chorda stimulation, and showed that it behaved in all physiological respects like acetylcholine. Similar demonstrations have been given by Bain (15) and by Henderson (16). A still closer correspondence of the liberated substance to acetylcholine is demonstrated in more recent papers by Feldberg (16a). Experiments by Gaddum and myself (17) brought this release of a substance, by impulses in parasympathetic nerve fibres in the neighbourhood of their endings, into clearer relation with certain other, abnormal effects of such nerves. Philippeaux and Vulpian (18) long ago showed that if the hypoglossal nerve was cut and allowed to degenerate, the muscles of the tongue acquired a new and abnormal reaction, responding by a prolonged contracture to stimulation of the chorda tympani. Sherrington (19) found that, when the motor fibres in the great sciatic nerve had degenerated, as the result of section of the corresponding anterior spinal roots, antidromic stimulation of the remaining, sensory fibres in the sciatic nerve caused a similar contracture of the denervated leg muscles. Heidenhain (20) had shown that the Vulpian contracture of the denervated tongue could be evoked by injecting nicotine; and, lastly, Frank and his co-workers (21) had shown that acetylcholine, in very small doses, produced similar contractures of denervated

muscles, and this action was related, by the observations of Professor Gasser and myself (22), to the secondary, nicotine-like action of acetylcholine. Now Hinsey and Gasser (23) had shown that the Sherrington contracture was due to the same class of sensory fibres as produced the antidromic vasodilatation of Stricker and of Bayliss;[2] so that, in the case of the chorda tympani and in that of the sensory supply to leg muscles, we were dealing with fibres normally vasodilator in function. Gaddum and I proceeded to investigate the possibility, already mentioned by Bremer (24) and by Hinsey and Gasser, that these abnormal contractures might be due to a substance normally functioning as a chemical transmitter of vasodilator impulses, and escaping by diffusion onto the adjacent voluntary muscle fibres, sensitized to its action by loss of their normal motor innervation. There was already much evidence, as we have seen, in favour of acetylcholine as the chemical transmitter of parasympathetic impulses, and the general features of the action of antidromic vasodilatation were in favour of its production by a similar mechanism. On the other hand, acetylcholine was the most powerful of known substances in producing contractures of denervated muscle. The suggestion, that it might be the agent responsible for the whole complex, was strongly supported by the fact that the effects produced by stimulating the nerves, like those produced by injecting acetylcholine, were intensified and prolonged by physostigmine. The difficulties in the way of attributing this function to acetylcholine were due to the effects of atropine, which readily extinguished the vasodilator effects of acetylcholine injected into the blood, but not those of stimulating the nerves; and to those of epinephrine, which readily suppressed the contractures in response to injections of acetylcholine, but might even intensify those produced by stimulating the nerves. The details of our investigations of the meaning of these discrepancies would make too long and too complicated a story. I must be content to give an idea of the conceptions to which they led us.

[2] A paper by Hinsey and Cutting (*Am. J. of Physiol.*, 1933, **105**, 535, published since these lectures were delivered, gives evidence that the fibres responsible for the Sherrington contracture originate in the sympathetic ganglia, and are not sensory. This seems to cancel the suggestion of the relation of the effect to antidromic action, but leaves untouched the evidence for its association with the release of a substance of the choline-ester type.

Acetylcholine injected into the blood-stream could only reach the voluntary muscle fibres from the blood flowing through the capillaries, by passing through their walls. Any hindrance to flow through the capillaries or lessening of their permeability might prevent its reaching the site of action; and epinephrine might produce both these effects. On the other hand if acetylcholine were liberated by nerve impulses on the muscle coats of the arterial walls, epinephrine would not affect its freedom of access, by diffusion through tissue fluid, to the voluntary muscle fibres; it might even, in that case, directly intensify its action. And we found, indeed, that, when acetylcholine was directly applied to an isolated strip of denervated voluntary muscle, the presence of adrenaline did intensify its action.

To remove the atropine discrepancy we must have recourse to a more speculative conception. We must suppose that the parasympathetic nerve endings have relations of differing intimacy with the reactive structures, so that the acetylcholine liberated at them must in some cases, as in those of the heart muscle fibres or the secretory cells, cross some gap or membrane at which atropine can impose a barrier, and in others, such as the plain muscle fibres of the arteries or of the alimentary canal, obtain such immediate access to the reactive structures that atropine cannot effectively interpose. It is, at present, not possible to put such a speculation to the test of experiment; but it seems to me easier to picture these varying actions of atropine along such lines, than to make them the basis of an assumption that the actions of parasympathetic nerves, everywhere reproduced with such fidelity by acetylcholine, except as regards this action of atropine, are in some cases mediated by liberation of acetylcholine, and in others by some substance that is not even a choline ester, or by a different, non-humoral mechanism.

Whatever view we adopt on that difficult matter, it will be clear, I think, that we have no physiological evidence of any actions of acetylcholine in the body, other than those produced by its local, peripheral liberation. Assuming it to be liberated as a peripheral transmitter of impulses in parasympathetic fibres, or of antidromic impulses in sensory-axon branchings to arterioles, we have insufficient evidence as to whether such impulses cause its immediate formation, by a synthetic esterification, or liberate it from an inactive complex, in which it is

held ready for their arrival. Though the latter, on general grounds, seems much the more likely, such evidence as we have from Professor Loewi and his colleagues is rather in favour of new formation. Of the vasodilator reactions in which we may suppose it to be concerned, some are localised to special organs receiving a true parasympathetic innervation. There is evidence, again, that certain fibres belonging anatomically to the true sympathetic system, such as those supplying the sweat glands, and those causing vasodilatation in the lips and buccal mucosa of the dog, may also act by liberating acetylcholine. Euler and Gaddum (25), in my laboratory, showed that the contracture of the denervated muscle in the dog's lip to stimulation of the cervical sympathetic nerve, as much earlier described by Rogowicz (26), is in every way parallel to the Vulpian phenomenon in the tongue, being apparently due to the liberation of a vasodilator substance of the choline-ester type, although the nerve fibres involved are anatomically sympathetic in origin and in ganglionic connection. The most important general vasodilator function of acetylcholine, however, if we accept the evidence which points in that direction, must be in connection with the arterial dilatation evoked by axon reflexes, through terminal branchings of sensory fibres in all parts of the body.

It may be of interest now to glance at the humoral mechanism of Lewis's threefold vascular reaction to skin injury in man, as it would appear in the light only of the evidence which we have so far considered. Histamine, liberated from epidermal cells by the changes following injury, will cause dilatation of the minute vessels lying immediately under the area injured, and local oedema by alteration of their permeability; at the same time, impulses starting from the sensory nerve-endings, initiated either directly by the mechanical injury itself, or, as Lewis suggests, by the action on them of the liberated histamine, will pass through the axon branching, down the side branch to a neighbouring arteriole, where liberation of acetylcholine will cause arterial dilatation, and produce the irregular flush surrounding the immediate injury reaction. So that the intervention of these two substances, liberated by entirely different mechanisms, at different parts of the vascular branching, would suffice by itself to account for the general features of the local vascular reaction to mildly injurious stimuli in certain species. It does not follow that they are the only

substances involved even in these cases; but before we leave them for a time, to consider other substances which may be concerned in such reactions, I should like again to emphasize the fact that what we know of the probable part taken by these two natural vasodilator substances, histamine and acetylcholine, in normal, physiological reactions, indicates that it is confined to the immediate neighbourhood of their liberation. Only under experimental, or otherwise abnormal conditions, can we detect effects of a more general kind, at a distance from the point of liberation, due to the escape of one or the other of these substances into the general circulation. And in this way the natural function of these vasodilator principles contrasts sharply with what is known of that of the true hormones, epinephrine and the post-pituitary principle, with their predominantly vasoconstrictor actions, produced simultaneously over the whole system if these substances are liberated into the blood.

Adenosine and its derivatives

Only some three and a half years ago a series of derivatives of the purine base adenine, already known to occur as natural constituents of various animal tissues, came first into view as substances having a pronounced pharmacodynamic action, of potential importance in the natural regulation of bodily functions and especially of the vascular tone. We may glance first at the formulae of some of the substances with which we are concerned (fig. 1). The purine base, adenine, might, as you will see, be thought to have a suggestive community of structure with histamine, in the presence of the glyoxaline nucleus. This, however, can have no significance for the activity with which we are concerned, since adenine itself is devoid of it. Condensation with a pentose, to form the nucleoside, adenosine, produces the activity; and so far as concerns its vasodilator action, in which we are chiefly interested, this is further intensified, for some species at any rate, by further condensation of the pentose group with a molecule of phosphoric acid, to form adenylic acid; and yet further again, by the union of adenylic acid with molecules of pyrophosphoric acid, to form the complex in which the substance appears naturally to occur in skeletal muscle, in which the form of its occurrence and its physiological significance have been chiefly studied. There is evidence for two different

kinds of change in the adenylic acid complex of muscle, occurring in muscular contraction: (1) the liberation of pyrophosphoric acid, and (2) the deamination of adenylic acid to inosinic acid, with reversible liberation of ammonia. For our purpose, the points of importance to note are that inosinic acid possesses none of the pharmacodynamic activity with which we are concerned, and that free adenylic acid is not more, but apparently somewhat less active as a vasodilator than adenylpyrophosphate.

The first evidence that the substances of this series contributed to the effects of tissue extracts on the circulation was obtained by Drury and Szent-Gyorgyi (27), who were seeking for a constituent of extracts from mammalian heart and skeletal muscle which produced, on intra-

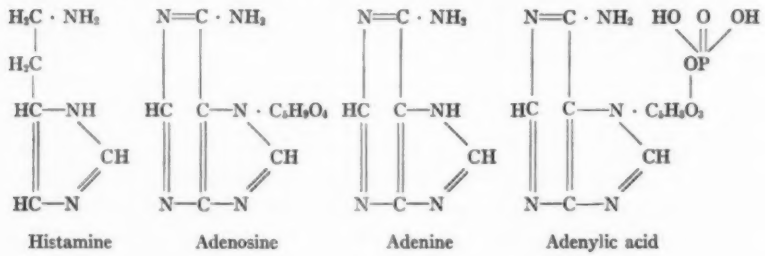

FIG. 1

venous injection, a characteristic condition of heart block, especially in the guinea-pig. The substance which they isolated proved to be adenosine, already known to be a normal extractive from brain, muscle and other tissues, and the natural complex, adenylic acid, was found to have a similar activity. Acid hydrolysis, however, splitting off the pentose and liberating adenine, destroyed the action. Later experiments showed them that these substances produced a fall of arterial pressure greater than would be expected from the slowing of the ventricular beat; and perfusion experiments confirmed the expectation of a general vasodilator action. This vasodilator action has later been the subject of investigation in different laboratories, and it is in relation to it that speculations have chiefly arisen as to a probable rôle of the adenosine compounds in connection with the control of the circulation.

As to the nature and location of the vasodilator effect, there is no

doubt that the arterioles are concerned. In some accounts of the probable function of these substances mention has been made of a dilator effect on the capillaries in addition; but I have seen no direct evidence in favour of this effect. A direct dilator action on the capillaries is, in any case, difficult to detect in the presence of an undoubted and strong dilatation of arterioles. Bennet and Drury (28) tested adenosine as Lewis had tested histamine, and found no evidence of local capillary dilatation and wheal formation, as the result of puncturing a solution into the human epidermis. The absence of such a capillaridilator effect, indeed, satisfied them that their preparation of adenosine was not contaminated by histamine, even to the extent of 1 part in 1,000. I think we may accept the view, on present evidence, that the effect of the adenosine compounds on the blood vessels is essentially to produce arterial dilatation. Their depressor action is very much weaker than that of the substances, histamine and acetylcholine, with which we have dealt already. It appears, rather, to be of the relatively weak order of that produced by free choline. This relative weakness, however, does not affect the possibility that these substances, present in such quantities as they are in certain tissues, may contribute significantly to natural vasodilator effects. This vasodilator action has a wider distribution than that of histamine on the organs of different species, extending particularly to the rabbit. It appears to be, indeed, more powerful in its dilating action on the rabbit's vessels than on those of the cat; so that, when a tissue extract produces a vasodilator fall of blood pressure in the atropinized rabbit, the adenosine compounds must be excluded as the primary possibility, before other active substances are considered; just as for such an effect in the cat, under similar conditions, the presence of histamine is the primary possibility to be excluded.

In some directions the actions of the adenosine group in the rodent would appear to be capable of partially replacing those of histamine in the carnivora. Thus, histamine dilates the coronary vessels in the cat and dog, but constricts them in the rabbit, whereas the adenosine compounds are powerful coronary dilators in all these species. They also dilate the arteries in the frog. The kidney vessels of all species seem to form an exception to those in the rest of the body, in that adenosine, like histamine, appears to act as an arterial constrictor in

that organ. Altogether, however, though adenosine and its derivatives fill an important gap in our knowledge, as being substances of known constitution, present in many tissue extracts, and producing a vasodilator fall of arterial pressure in species which do not give this response to histamine, their action is of a type so different from that of histamine that they can hardly be regarded as functionally replacing it in certain species; they must rather be considered as making a further and qualitatively different contribution to a complex effect.

As regards their distribution, this seems to be pretty general in the important organs; but those containing it most abundantly, according to Bennet and Drury (28), are the cardiac and voluntary muscles, the liver and the kidney. The skin and the lung, on the other hand, yield relatively little; so that, in general, the tissues richest in histamine appear to be relatively poor in the adenosine compounds, and *vice versa*. The blood yields little to extraction; on the other hand, the substance having a vasodilator action, appearing immediately in blood removed from the vessels, and referred to by Freund as "Frühgift," is stated by Zipf (29) to be adenosine or adenylic acid.

The action of these substances on the circulation has only been known during the past few years, and our data for judging the probabilities of their natural function are, of necessity, incomplete, in comparison with those which nearly a quarter of a century of detailed study has given us for histamine and acetylcholine. It is natural to think, in the first place, of their possible intervention as regulators of the blood flow through the voluntary muscle and the heart, in accordance with their states of functional activity. Unless the findings of Kapfhammer and his colleagues, as to the presence of large quantities of acetylcholine in these tissues, should be confirmed, the adenosine group are the principal vasodilator constituents which can be isolated from them as chemical entities. If we could assume them to be liberated from the muscle fibres, as a concomitant effect of contractile activity, and thus brought into contact with the arterioles, we should have a satisfactory explanation of the enormous increase of blood-flow accompanying, and especially following, the activity of a muscle, for which the hitherto suggested effects of lactic acid and carbon dioxide have never seemed really adequate. Present knowledge of the contractile metabolism of the adenylic acid complexes, however,

gives no direct support to such an assumption. Deamination deprives them of activity, and cleavage of the pyrophosphoric acid complex does not increase it. It is obviously not impossible that the larger complex may be held more firmly in the muscle protoplasm, and that some of the free adenylic acid liberated in contraction may escape to produce vascular effects (cf. Rigler (30)). The most, however, that can properly be said of this possibility as yet is, in my opinion, that the presence of these fairly potent vasodilator substances is not likely to be without significance, when we find them in organs which are subject to such a large functional vasodilatation, and which are not known to possess nervous or other chemical mechanisms adequate to account for it. Rigler (30) has shown, indeed, that in frog's muscle poisoned with iodoacetates and, therefore, no longer liberating lactic acid in contraction, contractile activity is still accompanied by a vasodilatation, comparable to that produced by adding a small dose of adenosine to the perfusion fluid. For more direct evidence, however, of the participation of these substances in producing the vascular relaxation which accompanies and follows activity, and as to the manner of this intervention, we must look to the future.

If we could still accept the experimental evidence for a chemical factor in the shock following extensive muscular injury, it would be natural, again, to consider the adenosine group in that connection. As we have seen, however, the validity of that evidence has been questioned. It seems to me premature to suggest for adenosine, as some observers are inclined to do, a predominant rôle in conditions of shock following extensive burns. The best experimental evidence is that produced by Bennet and Drury (28), who perfused a rabbit's heart, and delivered the effluent from it to the arterial cannula of a second heart, similarly perfused. When the first heart was subjected to heat injury, the second exhibited a complex of reactions, which they analysed into effects corresponding to those of adenosine and of histamine. It seems reasonable, indeed, to expect that if tissues are lethally injured by heat, under conditions which allow the products liberated from them to escape into the general circulation, any resulting vascular reaction will involve the effects of all the substances active on the blood vessels which the injured cells normally contain. It may be better, therefore, to postpone a further consideration of the more

general, pathological importance, which some would attribute to the adenosine group, until we have reviewed the evidence for the existence of yet other vasodilator constituents of the tissues. Another effect which some have attributed to liberation of adenosine or its derivatives is the reactive hyperaemia which follows renewal of the circulation after temporary arrest (Zipf (29)). The chemical origin of this effect has been experimentally traced by Lewis and his colleagues, and there is nothing known of the action of adenosine which is inconsistent with its participation. On the other hand, there is yet no complete evidence of its liberation under such conditions, and no ground for attributing to it an exclusive or predominant rôle in the production of this effect.

There is one action of adenosine, mentioned by Drury and his colleagues, which may well be of significance in connection with certain reactions to injury, namely, its power of inducing a leucocytosis. The fact that histamine has not this effect has always prevented the allotment to it of more than a partial function, in a vascular response to injury severe enough to be classed as inflammatory. Whether the combined effects of histamine and adenosine will suffice to account for the whole of a local reaction to injury, more severe than that required to liberate histamine alone, must be decided by further study.

It will be clear that enough is known already of the action of the adenosine series to justify the expectation that a natural function of importance will, in due course, be attributable to their vasodilator actions. It is a matter, meanwhile, for satisfaction, that another part of the complex vasodilator action of certain tissue extracts can now be attributed to a group of chemically defined substances.

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III. KALLIKREIN. VASODILATORS FROM BLOOD-VESSELS, BLOOD, INTESTINE AND BRAIN. GENERAL SURVEY OF THE POSSIBLE FUNCTIONS OF SUCH CONSTITUENTS OF THE TISSUES, IN RELATION TO THOSE OF NERVES AND SPECIFIC HORMONES

In my first two lectures I dealt with those of the vasodilator substances occurring in various tissues which have been chemically isolated and identified. We have the more difficult task to-day of considering the evidence for the existence in the body of yet other vasodilator principles, concerning the chemistry of which no such definite statement can be made. Claims for their existence as independent principles, separate from those that we have already considered and

from one another, must be based partly on their physiological effects. It will be clear, however, that when a substance has not yet been chemically identified, so that we have no adequate criteria for its purity, even the regular association, in the effects produced by different preparations of it, of certain physiological actions in constant proportions, does not give us a final certainty that we are dealing with the actions of one chemical individual.

Kallikrein

The highly active vasodilator substance to which Frey and Kraut (1, 2, 3) gave the name *Kallikrein* was first obtained by them from normal urine. Many years ago Abelous and Bardier (4) found a depressor principle in urine, which was not dialysable, was insoluble in alcohol, precipitated by ammonium sulphate and adsorbed by animal charcoal, and was obviously a fairly complex substance. Frey and Kraut separated what is probably the same depressor principle as Abelous and Bardier's "urohypotensine," though apparently in a less thermostable form, by applying the adsorption and elution technique of Willstätter. By treatment with a series of different adsorbents they have concentrated the activity into a relatively small weight of material (5). Kallikrein differs from all the other substances which we shall have to consider in its complexity and instability. It will not dialyse through parchment, and it is inactivated by boiling. It resembles in these respects some of the more complex hormones rather than the simple constituents of ordinary tissue extracts; and we shall see that something like a true hormone function has been attributed to it. It gives the ordinary colour reactions of proteins and their amino-acids rather weakly, but has never been wholly freed from them. In particular, it originally gave the diazo-reaction of Pauly with sufficient strength to suggest that histamine was present in it. The actions first described, on the heart-beat and circulation of the dog, were not unlike those of histamine, but were more intense than those which would have been produced by histamine, in a dose giving the same intensity of diazo-reaction. With further purification the activity has increased in relation to the weight, while the diazo-reaction has weakened rather than increased. While, therefore, it cannot be assumed that Kallikrein perfectly free from histamine has yet been

produced, the activity cannot be regarded as mainly due to such contamination. The purest specimens have, apparently, about one-tenth of the depressor activity of histamine in the dog, but give a diazo-reaction of only about one hundredth the intensity. The physiological effects, though superficially resembling those of histamine, in some respects appear to differ in significant detail. Thus the augmentation and acceleration of the heart-beat in the dog are larger in proportion to the depressor effect, in comparison with those produced by equi-depressor doses of histamine. According to Elliot and Nuzum (6), the augmentor effect on the heart is secondary to coronary dilatation, which Kallikrein produces in the isolated, perfused heart of the rabbit. The depressor activity of Kallikrein, moreover, is seen in the rabbit as well as in the carnivora. In stimulating the intestinal muscle of the guinea-pig, but not that of the rabbit, to contraction, Kallikrein resembles histamine; on the other hand, it inhibits the tone of the uterus of a cat, in contrast to the intense stimulant action of histamine. The dog's liver shrinks during its depressor action, whereas it swells during that produced by histamine. In the guinea-pig Kallikrein does not cause the constriction of the bronchioles which an equi-depressor dose of histamine would produce, and which forms such a characteristic feature of the anaphylactic shock in that species. Altogether the physiological actions of Kallikrein, so far as they have been analysed, are not compatible with a suggestion that it might owe its activity to histamine adsorbed on some more complex substance. They do not yet exclude, indeed, the possibility that histamine may make some contribution to the effects of the purest specimens of Kallikrein yet produced. As regards the main features of the action of Kallikrein, however, these do not seem to be explicable as a mixture of the effects of known principles; the whole activity may be due to one substance, of chemical nature not yet defined, or to more than one. The nature of the substance does not allow us yet to attribute even physiological purity to it.

Perhaps the most interesting properties of Kallikrein, however, are those associated with its occurrence in the body and its behaviour when added to body fluids. Blood serum has the power of rapidly inactivating Kallikrein added to it, and this effect is attributed by Kraut and Frey to the union of Kallikrein in definite combining proportions

with a specific inactivator (7). The inactivation occurs at the normal reaction of blood, but the union is broken, and active Kallikrein again liberated, when the reaction is made acid. Kallikrein is said to be present in blood in relatively large amount, but united with the inactivator; and it is suggested that acidity in a tissue, resulting from metabolic activity, can release it to produce the functionally appropriate vasodilatation. The kidney, it is suggested, dissociates the union with the inactivator, so that active Kallikrein passes into the urine. From various tissues Kallikrein, often associated with its inactivator, has been extracted; but, according to its discoverers, the pancreas yields much more than any other organ. Gley and Kisthinios (8) had also obtained from pancreas a substance of similar activity. Frey and Kraut believe (9, 10) that the pancreas has the function of forming Kallikrein, and that from this central factory and depot the blood carries it in inactive form, to exert its action when peripherally liberated. In support of this view they give evidence that removal of the pancreas causes the Kallikrein content of the urine, stated in activity per unit of dry weight, to fall very low (3, 9). Such evidence as this affords, in favour of the pancreas as the Kallikrein factory, seems to be seriously weakened by the further observation that insulin, in the absence of the pancreas, restores the normal excretion of Kallikrein.

I think it may fairly be suggested that confirmatory and additional evidence will be required before this theory of the origin and distribution of Kallikrein can be definitely adopted. We may note, however, that, even if it be accepted, Kallikrein, though centrally formed and generally distributed from a special organ, and therein resembling the true hormones, would conform in its mode of action to that of the other known vasodilator substances, in that its effects would be confined to the immediate neighbourhood of its ultimate liberation at the periphery, and not produced simultaneously throughout the vascular system. Kallikrein, as you will realise, is a principle definable as yet only by its physiological activity and the mode of its occurrence, rather than in chemical terms. Its thermolability, and the readiness with which alcohol precipitates it, will necessitate, for any further progress in the investigation of its chemistry, quite different methods from those that have been employed in the isolation of other vaso-

dilator substances from tissue extracts. The further studies which are promised may strengthen the evidence for its physiological importance; but its discovery does not add to our knowledge of the vasodilator constituents of ordinary alcoholic or boiled extracts of tissues. For additions to the list of such constituents, already containing histamine, choline and the adenosine group, we must turn to investigations of another kind.

Vasodilator substances of Euler and Gaddum, of Felix and Lange, and of Major

It will be convenient to consider together several further investigations, one carried out in my own laboratory by Euler and Gaddum (11), another in Munich by Felix and Lange (12, 13, 14), and a third by Major and his co-workers (15, 16, 17) in this country. It is by no means certain that these groups of investigators have all been dealing with the same substance; but they have had the common aim of filling the gaps still left in our chemical knowledge of the substances responsible for recognized types of vasodilator activity in tissue extracts. In the case of each of the substances already identified, there are certain combinations of physiological action which, as we have seen, can be regarded as diagnostic. There have been several indications of the presence, in certain tissue extracts, of another such association—that of a vasodilator action, effective in the rabbit as well as in the carnivora, with a potent stimulant action on rabbit's intestinal muscle. The resistance of both these effects to atropine excluded choline and its esters; neither the depressor effect on the rabbit's circulation, nor the stimulant effects on its intestinal muscle could be attributed to histamine; while the adenosine series inhibit the activity of rabbit's intestinal muscle, and, as we shall see, can further be excluded with some ease by chemical methods. The frequent occurrence of these effects, however, and even their continued association in apparently constant proportion, through various chemical manipulations, give us no certainty that they are due to one substance, unless a chemical individual should be isolated, and be found to produce both. The combination of effects has been used as a guide, however, in an attempt to find another vasodilator principle.

So far as I am aware, this particular association of effects was first

indicated by Jendrassik (18), who extracted a substance from brain which produced them. Many years earlier Swale Vincent and his colleagues (19, 20) had described a depressor effect on the atropinised rabbit, produced by brain extracts; and in more recent years Major and his colleagues have emphasised the presence of a depressor substance in brain extracts, distinguishable from histamine and choline, and, later, from the adenosine series as well.

The investigations of von Euler and Gaddum, and those of Felix, Lange and their co-workers have a number of points in common. Both appear to have used intestine as the most available source of material. Euler and Gaddum, using the fresh small intestine of the horse, scraped off the mucous membrane, which contained relatively little of the substance they were seeking, and extracted the muscular coat with acidulated alcohol. Felix and von Putzer-Reybegg, working in coöperation with Lange, have taken the view that their principle is obtained from blood-vessels; but for their actual large-scale work they used material described as "*Gekröse*," and Professor Felix informs me that this included not only mesentery, to which the name more strictly applies, but the whole of the intestine as well. In many of their features, including the physiological effects described, the substances obtained by the two sets of workers appear to be similar. The substance is unstable to alkaline, but relatively resistant to acid hydrolysis, by which adenosine can be eliminated. Euler and Gaddum could also get rid of adenosine by dissolving their preparation in strong alcohol, or by precipitating the active principle with Reinecke acid, and leaving the adenosine compounds in solution. Felix and Putzer-Reybegg claim that their principle is stable at 100°C. even with 30 per cent sulphuric acid; and if this is the case, they are dealing with a much more resistant principle than that studied by Euler and Gaddum. The adenosine compounds could, in either case, be eliminated without much difficulty, but it proved much more difficult to free either preparation completely from histamine. Felix and Putzer-Reybegg claim to have achieved this by destroying the histamine with nitrous acid. In any case, however, the two actions which have furnished the guide to the separation of the new substance or substances by Euler and Gaddum, the depressor effect on the atro-

pinized rabbit, and the stimulating action on the rabbit's intestinal muscle, which is also resistant to atropine, are not histamine effects.

There could be no doubt, therefore, of the existence in certain tissues of at least one vasodilator substance, differing in the details of its physiological action, and in some of its chemical properties, from any of those hitherto isolated, and also apparently different from the large-moleculed and thermolabile Kallikrein. Tracing the substance by its characteristic association of physiological effects, Euler and Gaddum found a curious distribution, in that they obtained much higher yields from the stomach and intestine and from the brain than from any other organ. From lung, liver, kidney, pancreas, spleen and skeletal muscle they obtained the characteristic activity only in small traces. The fact that Felix and Lange found evidence of a high yield of their principle from the kidney may be another indication that they are dealing with a different substance.

Euler and Gaddum's observation that the brain, and in particular the basal ganglia, gave extracts peculiarly potent in producing the syndrome characteristic of their substance, brings their findings into suggestive relation with those of Major and his co-workers, who have also found evidence of the presence in brain extracts of a principle dilating the rabbit's vessels, distinguishable from histamine and choline by its actions, and from adenosine by its stability to acid hydrolysis. On the other hand, Major's substance appears to differ from that of Euler and Gaddum by the fact that it does not stimulate the muscle of the rabbit's bowel. There is always a possibility, however, that Euler and Gaddum's purest preparations still contained two active substances, and that their methods had failed to separate them, or even perceptibly to change their proportions.

You will appreciate the difficulty of disentangling this evidence as to the nature of the substance or substances concerned in these three independent investigations, and of deciding even as to the probability of their being to any extent identical. We can, indeed, regard them all as confirming and reinforcing the evidence, previously available, as to the existence of one or more natural vasodilator constituents in certain tissues, in addition to those already identified. It is unlikely, however, that we shall be able to decide whether these

different investigators have been dealing with one or more than one such additional substance, or to appraise the likelihood of any of them subserving in the rodents a function similar to that which we can attribute to histamine in other mammals, until they can be obtained in a condition of chemical purity, and their detailed actions can be examined and compared, without any question of complication from the actions of other substances. Fortunately, there appears to be, from the latest results reported by two of these groups of investigators, some prospect that this may be realized. Felix and Putzer-Reybegg (21) report the isolation from their material, as crystalline flavianate, picrate and aurichloride, of an intensely active base, certainly different from any of the depressor bases already known, with a formula $C_4H_{12}N_6$. This base gives no Pauly diazo-reaction, but the Sakaguchi reaction, specific for arginine and guanidine compounds generally, with great intensity. The analysis, and the properties of the salts described, would be compatible with the substance being methyl-guanidine; but this would have none of the intense physiological activity attributed to the compound isolated. Diguanidyl-ethane, which has the same empirical formula, is a known substance, and its salts have quite different melting points from those observed. A compound formed by the asymmetrical union of two methyl-guanidine molecules would give a structure satisfying the formula; but such a compound seems unlikely to have the extraordinary stability attributed to the new base. We must wait for further evidence, and hope that it will enable us to add another to the list of intensely active tissue substances of known constitution.

Weber, Nanninga and Major (22) report the separation, from an alcoholic extract of brain, of a crystalline substance, very soluble in alcohol or water, but insoluble in ether. It is said to retain its activity on repeated recrystallization from alcohol, and to give neither the Pauly nor the Sakaguchi reaction, so that it is neither histamine nor a guanidine derivative. The activity reported is not a high one, in comparison with that of histamine, for example, since half a milligramme was required to produce a fall of 40 mm. in a dog's arterial pressure. Atropine does not prevent the action, but no evidence is given as to the effect on the rabbit's circulation. It is not quite clear, therefore, whether the crystals represent in pure form the sub-

stance with which the earlier papers of Major and his co-workers were concerned. Again we must wait for further evidence as to the chemical nature of the substance, and as to the details of its mode of action.

Altogether, then, the position as regards the identification of other vasodilator principles from tissues, to fill the gaps which were left after the identification of choline, acetylcholine, histamine and adenosine, is still one of hopeful prospect rather than final achievement. The difficulties increase at each further stage of progress, and it is a matter for satisfaction that workers in several different countries have interested themselves in the attempt to complete the story.

Hitherto we have spoken only of tissue principles, which, so far as their action on the circulation is concerned, produce effects of a depressor, vasodilator type. And these, indeed, so predominate in the action of any ordinary tissue extract, other than those from special endocrine organs, as normally to mask the effect of any pressor, vasoconstrictor substance which might be present. The presence of such pressor substances has been detected, however, by special methods in a variety of tissue extracts, and in a number of investigations, beginning many years ago with those of Vincent and Sheen (19). The clearest evidence is that given more recently by Collip (23), who found a pressor principle which passed, from a strongly concentrated watery extract of almost any organ, into a layer of acetone and ether, leaving the depressor constituents in the concentrated watery decoction. The general type of the action appeared to be sympathomimetic; I myself found it to be reversed by effectively paralytic doses of ergotoxine. We know practically nothing of its chemistry, and it has apparently not been further investigated. Collip raises, quite properly, the question whether this pressor substance is a natural constituent of the tissues, or is produced by the process of extraction; but I think it can fairly be said that there is no step in his process which could be suspected of artificially producing an active substance, not potentially present in the living cells. On the other hand, there is nothing against the probability of its normally occurring in an inactive complex, and being liberated therefrom by the artificial conditions of extraction.

Collip's principle shows a suggestive resemblance in its action to a substance which appears in blood removed from the vessels. The

appearance is apparently favoured by mechanical agitation, as by whipping, but the activity appears even in blood prevented from clotting by heparin, and allowed to stand quietly in a glass vessel. Freund (24) has distinguished, in shed blood, the action of a depressor, "*Frühgift*," appearing immediately, and stated by Zipf (25) to be adenosine or adenylic acid, and a later appearing "*Spätgift*," with vaso-constrictor action—a substance familiar to all who have experimented with the perfusion of living organs artificially with defibrinated blood. Recently Heymans (26) has shown that the action of this vaso-constrictor substance, appearing spontaneously in shed blood, is reversed by ergotoxine, like that of epinephrine. We are tempted to speculate whether any of the epinephrine which the blood carries from the suprarenal medulla may be held in some large, inactivating complex, disintegrated by artificial contacts to an extent allowing the epinephrine action to become partially effective. The question of the nature of the physiologically active substances, which appear in shed blood, is a large and complex one in itself. The fact, however, that such a change of conditions, as the shedding involves, suffices to bring into action a series of substances which the blood must hold inactive under the conditions of normal circulation, may, I think, legitimately encourage us to believe in the natural liberation, under changing physiological conditions, of the substances which chemically conservative processes so readily extract from other tissues.

That brings us to what is really the central and difficult problem, presented to our physiological judgment by the observations with which I have been dealing in these lectures. How far does the extraction from the tissues, of substances showing physiological actions when artificially injected or applied, justify us in assuming that those substances, by virtue of these actions, intervene in normal physiological reactions? For obvious reasons it is very difficult to obtain direct evidence in answer to such a question, in the case of many of the substances with which we have dealt. In the cases of histamine and acetylcholine, indeed, we have seen that, under conditions of physiological reaction, in which the liberation of one or the other had been suggested as the effective cause, direct evidence has been obtained of a substance with corresponding actions and properties in the fluids leaving the reacting tissue. It has taken nearly a quarter

of a century, however, to make this approach towards certainty in the case of substances so long known, and many years of investigation may be required before a physiological function can be as closely defined, and suggested with equal confidence, for the substances which have come more recently into view.

I have already alluded more than once to the fact that the substances, widely distributed in the tissues, which have provided my subject, have been recognised primarily by their actions on the circulation, and in particular on the blood vessels, and that this action has in almost all cases been to cause vasodilatation. There has been a tendency for attention to be centred on this one of their actions, though several of them produce powerful and striking effects on the plain muscle of other organs, on gland cells, and on the heart. I do not think that this choice of the action on blood vessels for special attention can be regarded as arbitrary, or merely as one of convenience. I think it represents rather a recognition, perhaps subconscious, that the one reactive system with which all these substances will come into immediate contact, at the site of liberation from the tissue cells containing them, is that of the smaller blood vessels. And I think that what evidence we have, as well as general considerations of probability, suggests that such natural actions as we can attribute to these substances are mainly localised to the immediate neighbourhood of their release. The conditions of their liberation vary of course. The experimental evidence which we have as to the liberation of acetylcholine in the body, suggests that this occurs only at the call of impulses in parasympathetic nerve fibres, or in the functionally equivalent branches to the arterioles from sensory fibres. Any traces of what is released by this mechanism, escaping into the bloodstream, must be entirely ineffective under normal conditions. Histamine, released by injury or irritation of cells containing it, exhausts its effects normally on the immediately neighbouring capillaries and minute arterioles. In 1919 I spoke here of a possibility, unsupported by evidence, that histamine, or something like it, might be released by functional activity of an organ, to cause local vasodilatation in accordance with an increased metabolic need. No evidence has presented itself in the interval to support that idea; and the fact that the tissue in the body showing the widest and most sudden fluctuations of

metabolic demand, namely, the voluntary muscle, contains the least histamine of all the tissues examined, definitely discourages it. Two new claimants have appeared, however, for this function of locally regulating the blood-flow, by vasodilatation in proportion to metabolic activity, namely, the adenosine compounds and Kallikrein. Their claims for the moment must be granted a greater plausibility, but the future must show whether they will bear a critical scrutiny, as severe as that by which the claims of histamine to this function have already been reduced to that of, at most, a possible accessory. Similarly, the claim put forward both for adenosine and for Kallikrein, as the effective vasodilator agent in the so-called reactive hyperaemia—the wide vasodilatation observed in an area to which the blood is readmitted after temporary arrest of the circulation—this claim is, in either case, simply one deserving examination, and requiring further evidence. Even a plausible suggestion of a natural function for the other, partially or completely separated tissue-vasodilators, can hardly yet be made.

It seems to me that we need much more direct evidence, before we can confidently attribute to any of these vasodilator substances a pathological production of general vascular collapse, due to its being released into the blood-stream, and thus producing its effects simultaneously over the whole body. When tissue injury is general, as in the anaphylactic reaction, I think that we have reason to postulate a general release of histamine, with widespread symptoms corresponding to its action; and it is certainly possible that others of these active substances may also be generally liberated under such conditions. But for their distribution from sites of local injury, to become the essential factors in an ensuing shock, I think we still lack evidence. We may regard these natural vasodilators, then, whether liberated by nervous action, by injury, or by metabolic activity, as normally effecting a local regulation of the blood supply. There is, one may suggest, no probable physiological requirement for a relaxation of the blood-vessels in all organs and tissues simultaneously. A physiological enhancement of general vascular tone, on the other hand, is a physiological requirement for which the body has at least a double provision, in the activation of sympathetic nerves by the vasomotor centres, and in the nervously regulated output of epinephrine. As

to the pressor hormone of the posterior pituitary lobe, we know too little of the conditions regulating its output into the circulation, or as to whether, indeed, it acts chiefly through this channel, or by directly affecting nerve centres in the floor of the third ventricle, as Professor Cushing's (27) striking clinical observations suggest.

The suggested contrast between vasomotor effects, on the one hand, produced generally by the action of vasomotor centres or by blood-transport of true, vaso-constrictor hormones, and vasodilator effects, on the other, produced locally in accordance with detailed requirements by local release of vasodilator substances, is not absolute. Since the curious action of ergotoxine first came to light, I have believed in a normally submerged vasodilator component in the effects of sympathetic nerves and epinephrine; and the clinical observations of Lewis and his colleagues, on patients subjected to sympathectomy, appear to prove its existence in man. Broadly, however, I think that the contrast holds good. At a time when our interest in the vasodilator substances from tissues was chiefly directed to the action of histamine, I drew attention to the antagonism between its effects, in various directions and those of epinephrine. Removal of the suprarenal glands from a cat was followed within 24 hours by an enormous increase of sensitiveness to histamine, of which a dose normally causing only a transient depression would now produce an irremediable shock-like condition, with loss of a large part of the blood plasma from the vessels (28). The recent observations of Swingle and his coworkers (29) reinforce the suggestion, made by me at that time, that loss of the cortex was somehow concerned in this complete lack of resistance to histamine, but, as Kellaway and Cowell (30) subsequently showed, destruction of the medulla alone causes an increased sensitiveness to small injections of histamine, though not of the extreme kind seen after total adrenalectomy. It is easy to show that the output of epinephrine is accelerated in response to small injections of histamine and acetylcholine. Possibly such an output occurs in response to the effects of others of the natural depressor substances. For Kallikrein a more direct antagonism to epinephrine is suggested. It is stated that a dose of epinephrine injected during a Kallikrein depression has a relatively small and brief pressor action. Elliot and Nuzum (6) state that repeated doses of Kallikrein will

produce a temporary obliteration of the pressor action of epinephrine, persisting even after the circulation has recovered from the Kallikrein depression.

I was formerly tempted to suggest for epinephrine, apart from its function of adjusting the circulation and other bodily functions to the call of emergency, a constant protective action against the escape into the general circulation of naturally liberated quantities of histamine. If there were anything in this suggestion, it obviously ought now to be extended to include protection against other natural vasodilators. The provision by Nature of a double mechanism for producing the same effects, directly through sympathetic nerve impulses and indirectly by sympathetic release of epinephrine from the suprarenal medulla, has always suggested that the maintenance and adjustment of this control must have a particular vital importance. And now that the work of Loewi (31), and more recently of Cannon (32, 33), has shown that the effects of sympathetic nerve impulses are themselves transmitted by peripheral release of something like epinephrine (sympathin), the significance of the double mechanism seems to be emphasized. But I am bound to admit that the recent evidence does not seem to me to support the suggestion that a constant supply of epinephrine is needed to control and limit the effects of histamine and other vasodilators. Cannon and his colleagues (34) have now shown that a cat can lose, at successive operations, the suprarenal medulla, the entire sympathetic system, and the pituitary posterior lobe in addition, and still retain practically normal health, under the quiet conditions of laboratory life. It seems quite certain, therefore, that epinephrine is not normally needed to check, and to keep within physiological limits, the vasodilator effects of the tissue constituents which we have been studying. One would like to know, indeed, what would be the reaction of Professor Cannon's remarkable cat to a small injection of histamine; but we already know one thing quite definitely about it, namely, that without any sympathetic system, or suprarenal medulla, it was nevertheless capable of recovery from a further operation, which could hardly be performed without a fairly large amount of tissue injury. So that there is little basis left for the conception of epinephrine as the guardian of a healthy vasodilator tone, against the effects of vasodilator substances liberated from the tissues by the accidents and activities of daily life.

When the sympathetic nerves to a limb of a normal animal are cut, there is an immediate loss of tone in its vessels. Even within an hour, however, a new tone, not supported by nerve-impulses, has appeared; and formerly we should have attributed this recovery to the circulation of vasomotor hormones in previously ineffective concentration. Cannon's experiments seem to have cut us off from recourse to this plausible explanation. Yet the vessels of his cats must have a tone sufficient for a healthy circulation, though it cannot, indeed, rise to an emotional emergency. So far as our knowledge of possibilities goes, this tone must be entirely dependent for its local functional variations on the release of one or more of the vasodilator tissue-constituents which we have been considering. Yet it must be able to recover from such relaxation, and must be again maintained after restoration. What, then, is the source of this adequate tone, in a vascular system deprived of the known and normally effective nervous and chemical means of maintenance, but still exposed to the normal possibilities of depression by these generally distributed vasodilator agents? Have the vessels themselves a function of spontaneous tone in response to the pulsatile flow of blood through them, only displayed when the normal extrinsic control is removed? Or is some other, more generally distributed chemical agent of vasoconstriction, such as that extracted by Collip, effective under such conditions? The question can only be asked now; the future, perhaps, may answer it.

There is one other question, of more general application, which we ought to consider briefly in conclusion. I have already suggested that the discovery, in an artificial extract from an organ or tissue, of a substance which, on artificial injection, produces a pharmacodynamic effect, provides only a first item of presumptive evidence, in support of a theory that the action of this substance plays a part in normal physiology. Much more evidence is required before we can attribute clearly defined functions to such a substance, as we can now do in the cases of histamine and acetylcholine. But even where this is already possible, we have still no evidence to justify the assumption that the substance comes naturally into action in the body, in the free condition in which we isolate and identify it in the laboratory, after various unnatural chemical procedures. On the contrary, the experience gained in such chemical studies, of the tenacity with which such a

substance may cling to others of larger molecular dimensions, and of the degree to which its chemical properties may be obscured by such association, justifies a doubt as to whether any of these potent substances normally leaves the cell protoplasm in free solution. In our work on the isolation of histamine, we encountered preparations having a potent histamine action, but differing from the free substance in solubility, and even, in some cases, in failing to give the characteristic diazo-colour-reaction. We might easily have concluded that we were dealing with a substance of an entirely different and more complicated chemical nature, resembling histamine only in physiological action, had it not been for the fact that we found a method by which histamine could eventually be separated from this molecular association, and isolated as a pure salt. Such an experience of the difficulty of separating histamine, even by the relatively drastic action of laboratory solvents, from a complex which not only contained it, but allowed it to exhibit its physiological activity, makes it easy to believe that histamine may leave the living cell in molecular associations of varying complexity, which may modify to varying degrees its diffusibility, and obscure its chemical properties, while allowing its physiological effects to be produced. And the other principles which we have been considering may similarly come into action attached to relatively large molecular complexes, which may even contain more than one of these active substances. Such possibilities are hardly open to exact chemical study; it is only by the isolation of the pure active substances, and by study of their separate actions in detail, that we can ultimately identify the factors concerned in these complex physiological reactions. But it is important to remember that our procedures in the chemical laboratory often have a very distant relation to the processes of the living body; and, especially, that the occurrence, in some fraction of a tissue extract, of activity resembling that, say, of histamine on one organ, and that of adenosine on another, does not by itself entitle us to assume that we are dealing with a new kind of active principle.

If we consider the nature of the evidence, it must be admitted that we do not even know whether epinephrine, the secretion of which into the blood from its endocrine source can be so clearly and directly demonstrated, enters the circulation as a simple solution of the base

which we can isolate in the laboratory. There have recently been suggestions to the contrary from more than one source. Szent-Györgyi and his co-workers in Szeged (35) thought that they had demonstrated that epinephrine exists in the suprarenal medulla as a complex having many times the specific activity of the epinephrine which it contains. Dr. Schild in my laboratory has failed to confirm these results, and I think that he has detected the cause of the disparity on which the Hungarian authors based their conclusions. But we still do not know whether epinephrine as we know it in the laboratory, which requires a relatively drastic treatment for its first separation from a suprarenal extract, represents the hormone in the form in which it comes naturally into action.

While, therefore, the detection of a resemblance in physiological action does not, by itself, afford safe ground for an assumption that a known substance is present in a body fluid or a tissue extract, an equal caution is required before apparent differences in physical and even in chemical properties are accepted as final evidence for its exclusion. Such considerations do not tend to diminish the complexities of a difficult line of investigation, and I think we need not feel dissatisfied with the progress which has already been made. If we add, to the evidence with which we have been chiefly concerned, that which shows that effects of true sympathetic nerves are also transmitted to the effector cells by liberation of a chemical stimulant, in this case resembling epinephrine, it already seems possible to state that the whole of the control of the activity, not only of the blood vessels, but of all the involuntary muscle and gland cells in the body, is effected by a series of chemical, pharmacodynamic agents. These may be liberated by impulses in sympathetic or parasympathetic nerve fibres, including with the latter the axon-branches of sensory fibres to blood-vessels; or they may be formed in endocrine organs, and distributed by the blood to produce their effects simultaneously on all cells responsive to their action; or, finally, they may be widely distributed in tissues having no special endocrine function, and their liberation to produce local pharmacodynamic effects may be independent of any nervous control. Already we can write whole chapters of this story in terms of exact chemistry, and there can be little doubt that others will follow. Pharmacy, as the expert supply service of therapeutics,

will have to wait until the effect of this autopharmacology on therapeutic practice has more fully disclosed itself. It may well be that it will influence therapeutics more by the light which it may eventually throw on the causes of abnormal conditions, than by directly providing materials for their treatment. There are already plenty of tissue extracts available, with claims for their value in relieving a generally high blood-pressure, or a vascular spasm in particular organs; but it is to a completion of our knowledge of the active substances which they contain, of the details of the action of each, and of the relation of such actions to the physiological changes in the living body, that we must look, for the development of "a more intimate relationship between chemistry and medicine," and eventually between them and pharmacy, in this important field.

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CALCIUM AND PHOSPHORUS STUDIES

VII. The Effects of Variations in Dosage of Parathormone and of Calcium and Phosphorus in the Diet on the Concentrations of Calcium and Inorganic Phosphorus in the Serum and on the Histology and Chemical Composition of the Bones of Rats[1]

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It is now well established that hyperfunction of the parathyroids is associated with (*a*) an elevation in the concentration of the serum calcium, (*b*) an increased excretion of lime salts, and (*c*) osseous decalcification. The decalcification is usually followed by fibrous replacement or cystic degeneration, originally described by von Recklinghausen under the name of *osteitis fibrosa cystica*. In dogs (1, 2) and in guinea-pigs (3) such osseous and metabolic changes have been produced experimentally by the injection of a potent extract of the parathyroids. However, the experimental production of hypercalcemia or osteitis in rats is still a subject of controversy. Collip (4) and Greenwald and Gross (5) were unable to produce dangerous hypercalcemias in rats by parathormone injections and thought that these animals were immune to the effects of the hormone. On the other hand, Bauer, Aub and Albright (6), Tweedy and Chandler (7) and Cameron and Williamson (8) report moderate hypercalcemias in rats when large doses of the hormone were administered. Hypercalcemia and death in normal rats and in rats fed on diets deficent in vitamin B were also produced by Rose and Stucky (9) with similar doses of parathormone. Controversies also exist as to the effects of parathormone on the osseous tissue of the rat. Lambie, Kermack and Harvey (10) injected two rats with 10 units of parathormone daily for a period of twenty-one days and

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found that the bones of the treated animals contained less ash than normal but a greater percentage of calcium in the ash. Histologically, the bones appeared decalcified—this in spite of the increased calcium content of the ash. Waltner (11) described osteoporosis in growing rats kept on a stock diet and injected with 20 units of parathormone daily from 8 to 18 days; and Johnson (12) claims to have produced lesions typical of osteitis fibrosa cystica in rats six to twelve weeks old by the injection of large doses of parathormone. Bauer, Aub and Albright (6) found that, in contrast to the diminution in size and number of trabeculae of the bones of cats injected with parathormone, the bones of rats so treated were short and stubby and showed increased trabeculation. Histologic examination of the bones, however, was not made. The more recent observations of Selye (13) also demonstrate that an increase in the number of trabeculae is not an uncommon finding in rats injected with parathormone.

The apparent conflicting results become even more confusing because of the fact that, in several of the investigations mentioned, the composition of the diets is not given and hence they may not have had the same mineral content. Moreover, the daily doses of parathormone were varied during the experimental period. Thus, for example, although Johnson's animals were kept on the Steenbock stock diet throughout the period of observation, the variation in the daily dosage of parathormone and the introduction of large doses of viosterol in the diet during the period of parathormone injections makes his "very uniform results" somewhat complicated. In Selye's description of the experiments resulting in hypercalcification, no data are given in most instances as to age and number of animals, the diet, the exact dose of parathormone, and the duration of the periods of administration. The apparent contradictions throughout his papers may have been due to variations in the factors just mentioned. Thus, according to him, parathormone produced rickets in ten-day-old animals within six days (14); stimulation of bone formation in fourteen-day-old rats within forty-eight hours; hypercalcification in a 60-gram rat, and degenerative changes in older (?) animals (13). In the more recent experiments of Bülbring (15) adequate data are given as to the dietary calcium and phosphorus, dose and duration of parathormone, as well as histological and chemical analyses of the bones but, unfortunately,

her conclusions are based on the average results obtained under different conditions of experiment. Thus, for example, in comparing the percentage of calcium in the bones of animals treated with parathormone to those of the controls, the average for the entire parathormone group is used as indicating the decalcifying effects of the hormone; whereas, in reality, the individual analyses clearly indicate that the percentage of ash in the bones varied with the dose of parathormone employed. Small doses produced no significant alteration in the calcium percentage of the dry extracted bones, while large doses reduced the calcium of the bones quite materially. Her results are also somewhat complicated by the addition in the diet of too liberal amounts of vitamin D, in the form of 3 to 4 per cent cod-liver oil and 18 per cent butter fat.

The divergent results of the various investigations, therefore, may not be entirely due to faulty observation and interpretation, but rather to differences in the conduct of the experiments, especially with respect to the daily dosage of parathormone and variations in the calcium and phosphorus content of the diet—the latter being a factor frequently neglected in vitamin D and parathyroid studies. The necessity of controlling not only the dietary calcium but also the phosphorus in experiments dealing with calcium metabolism has already been stressed previously by Shohl and Bennett (16) and by us (17); and it became evident again during the course of study on the relation of vitamin D to the parathyroids. It was found that, in young rats fed rachitogenic diets, the injection of 0.1, 1.0, or 10.0 units of parathormone daily does not prevent nor cure rickets but, on the contrary, it intensifies the process in the rachitic metaphyses. Moreover, large doses of the extract may hinder the antirachitic activity of viosterol, *i.e.*, instead of vitamin D being identical with parathormone or acting only through the parathyroids, as suggested by Taylor (18), the functions of the two may actually be antagonistic. When we attempted, however, to produce either rickets, osteoporosis or osteitis fibrosa by giving 10 units of parathormone daily to animals fed the stock diet, the results were contrary to our expectations. The bones, instead of appearing decalcified, were actually harder than normal and contained an increased number of trabeculae which were compact and extended for some distance into the marrow cavity. These apparently divergent

results obtained in animals of similar age and treated with the same dosage of parathormone suggested that the difference might be due to the variations in the amount of phosphorus and in the calcium:phosphorus ratio of the respective diets employed. In the experiments to be described, an attempt was made to study the effect of variations in the amounts of calcium and phosphorus in the diet and of parathormone dosage on rats.

EXPERIMENTAL

Young growing rats, the offspring of healthy stock reared in our laboratory on the Steenbock-Bills diet (19), were used throughout this study. They were placed on the respective diets when they were eighteen to thirty-one days old and weighed about 45 to 60 grams. At least three rats were used for each experimental group, and in many instances the group-experiments were repeated. The parathormone (Lilly),[2] in amounts varying from 0.1 to 20 units daily, was injected intraperitoneally. *The same dose was used daily for the same animal throughout the period of observation.* The lengths of the periods of injection are given in the tables. In general, if the animals survived, the period of observation was 21 days, but in a few instances it was longer or shorter. The composition and the calcium and phosphorus content of the diets are shown in table I.

The animals were kept in wire mesh, raised bottom cages, and they were weighed at frequent intervals. Food and distilled water were allowed *ad libitum*. At the expiration of an experimental period the animals were bled, autopsied, and the soft tissues examined for gross changes and the presence or absence of metastatic calcification. A femur from each rat was freed of soft tissue, cut longitudinally, weighed, desiccated at 105° to constant weight, extracted in ether and alcohol, reweighed, and slowly ashed to white heat in an electric oven. The ash was weighed, dissolved in dilute acid, and made up to volume. The solution was then analyzed for calcium and phosphorus. The tibiae of each animal were used for x-ray and histologic examinations. The blood serum was analyzed for calcium and inorganic phosphorus. Data were thus obtained as to the weight and state of health of the

[2] We are indebted to Eli Lilly and Company, Indianapolis, Indiana, for their generous supply of parathormone

animals, the calcium and inorganic phosphorus of the serum, and the histology and x-ray appearance of the bones as well as their ash and calcium and phosphorus content. Uninjected animals of approximately the same age and weight, kept on the same diets and for the same length of time, served as controls.

TABLE I

Composition of diets

1. Stock diet (Optimal Ca and P)

Yellow corn	76.0
Crude casein	5.0
Linseed oil meal	16.0
Alfalfa meal	2.0
NaCl	0.5
$CaCO_3$	0.5

3 parts diet + 1 part powdered whole milk
Ca = 0.475; P = 0.450 gram per cent
Ca:P = 1.1:1

2. High Ca-low P (Steenbock rachitogenic)

Yellow corn	76.0
Gluten	20.0
NaCl	1.0
$CaCO_3$	3.0
	100.0

Ca = 1.240; P = 0.250 gram per cent
Ca:P = 4.9:1

3. High Ca-optimal P (Steenbock + 1.74 grams per cent Na_2HPO_4 anhydrous)

Ca = 1.240; P = 0.620 gram per cent
Ca:P = 2:1

4. Optimal Ca-high P (Stock + 3.00 grams per cent Na_2HPO_4 anhydrous)

Ca = 0.475; P = 1.100 grams per cent
Ca:P = 1:2.3

5. Optimal Ca-low P (Steenbock with only 1.0 gram per cent $CaCO_3$)

Ca = 0.440; P = 0.250 gram per cent
Ca:P = 1.8:1

6. Low Ca-low P (Steenbock with 0.5 gram per cent $CaCO_3$)

Ca = 0.250; P = 0.250 gram per cent
Ca:P = 1:1

7. Very low Ca-low P (Steenbock with only 0.1 gram $CaCO_3$ and 5 per cent fat-free yeast)

Ca = 0.100; P = 0.280 gram per cent
Ca:P = 0.35:1

RESULTS

1. *Stock diet.* The most striking effects on the osseous tissue were noted when 10 and 20 units of parathormone were injected daily. With the former amount, injected for either fourteen or twenty-one days, the changes may be described as follows: the bones appear hard and sclerotic and cut with difficulty. The osseous trabeculae of the spongiosa are dense and compact and extend for quite a distance from

the epiphyseal cartilage into the marrow cavity. These areas cast a dense shadow in the x-ray films (fig. 1, 3084–3059). *Histologically*, the disc of epiphyseal cartilage seems wider than normal, although the provisional zone is well calcified. Spicules of calcified cartilage extend for some distance into the shaft and show no evidence of bone formation about them. The trabeculae shaftward of this area are unusually numerous and compact and extend far into the shaft, invading and obliterating a large portion of marrow cavity (fig. 2). These trabeculae are also very cellular and contain large-sized, large oval and round cells with large nuclei, and because of their size and number reduce the ground substance between them. The interstices between the trabeculae are filled with reticular tissue which contains very few marrow cells but a large number of mononuclear cells. These cells are quite numerous at the margins of the trabeculae and seem like osteoblasts, many of which are in mitosis. Osteoclasts are much fewer than in normal bone. The periosteum is extremely cellular and the endosteal layer is composed of masses of osteoblasts. Fibrosis is also present here and there in the hyperostotic areas but it is extremely slight compared with the increased trabeculation and calcification.

The changes in the epiphyses are similar to those found in the shaft and are exaggerated in the epiphyseal center.

Ten units of parathormone daily thus induced in these animals a picture resembling osteosclerosis, *i.e.*, increased osteoblastic, as compared with osteoclastic, activity, persistence of the old calcified trabeculae and constant formation of new ones. The mass of old and new trabeculae produced the compact area beneath the cartilaginous zone which appears in the x-ray as a dense shadow. Similar bone formation, induced in animals by feeding elementary phosphorus, is usually termed *osteosclerosis*.[3]

With 1.0 unit of parathormone the osseous changes were identical with those described above except that they were present to a much lesser degree. The trabeculae were not as numerous—extending only for a shorter distance into the marrow cavity—and the osteoblastic activity not as pronounced. The bones of animals receiving 0.1 unit of parathormone daily differed little from those of normal control ani-

[3] Osteosclerosis and hyperostosis are used interchangeably throughout the paper. The latter probably describes the increased bone formations much more accurately.

mals, except for a very slight increase in the number of the trabeculae in the subcartilaginous zone. The amount of sclerosis was thus roughly proportional to the dose of parathormone, the greatest amount occurring with 10 units and the least with 0.1 unit daily (table II).

When the dosage of parathormone was increased to 20 units daily for 50 to 60-gram rats, the animals fared very poorly. Some died within four to five days and the rest were killed in a state of emaciation at the end of four to fourteen days. The bones of these animals were soft and broke and cut with ease. The x-rays of their bones showed extreme decalcification, areas of focal rarefaction, and areas in which the cortex was expanded by uncalcified material, so that they appeared cyst-like in the x-ray films (fig. 1, 3346–7–8). Histologically, the bones presented a most severe destructive process. The metaphyses were narrow and the trabeculae of the spongiosa were present only in places and were surrounded by much fibrosis. The cyst-like and rarefied areas noted in the x-ray films, likewise contained fibrous tissue (fig. 3). The marrow spaces were much encroached on and the marrow was, in a large measure, replaced by fibrosis. In many instances hemorrhagic areas were also noted in the areas of fibrosis and in the still-intact marrow. Osteoclasts were large and numerous and, apparently,

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FIG. 1. X-ray appearance of the bones in the different groups. The numbers correspond to the rat numbers in the tables

No. 3085, control, stock diet; nos. 3084 and 3059, stock diet and 10 units of parathormone daily; nos. 3346–7–8, stock diet and 20 units parathormone daily. Note the hyperostosis in 10 unit group and the extreme decalcification in the 20-unit group, especially the cyst-like area in the femur of no. 3347.

No. 3339, control, Steenbock diet with 0.5 gram per cent $CaCO_3$; no. 3342, same plus 10 units parathormone daily. Note the wide metaphysis and the submetaphyseal sclerosis.

No. 3117, control, Steenbock diet + Na_2HPO_4 (calcium:phosphorus = 1:0.05); no. 3000, same plus 10 units parathormone daily.

No. 3581, control, stock diet + Na_2HPO_4; no. 3571, same plus 10 units parathormone daily. Note the areas of decalcification as contrasted with the sclerosis in nos. 3084 and 3059 (stock diet).

No. 3068, Steenbock diet and 0.1 per cent viosterol 1-D. Note absence of rickets.

No. 3108, Steenbock and 10 units parathormone daily. Note absence of healing in the provisional zone of the metaphysis but moderate sclerosis just below the metaphysis.

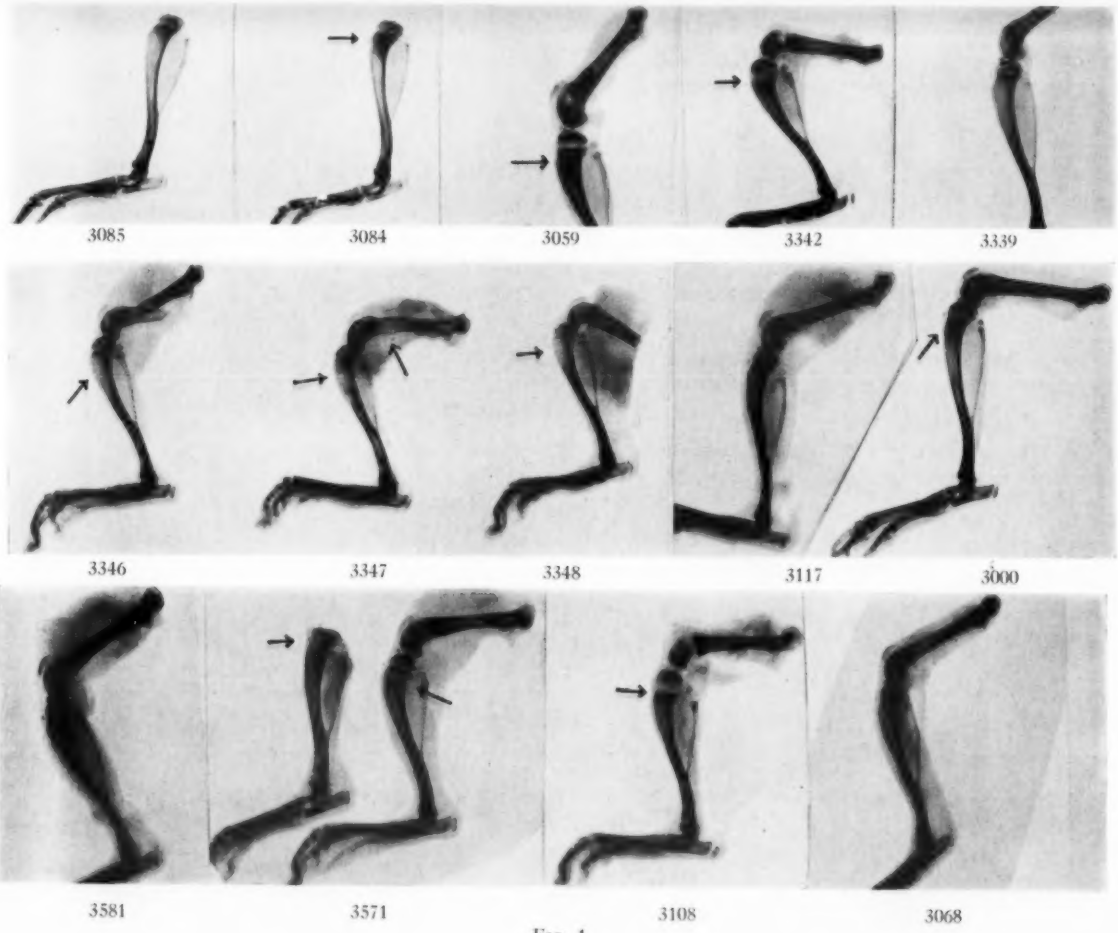

3085
3084
3059
3342
3339
3346
3347
3348
3117
3000
3581
3571
3108
3068

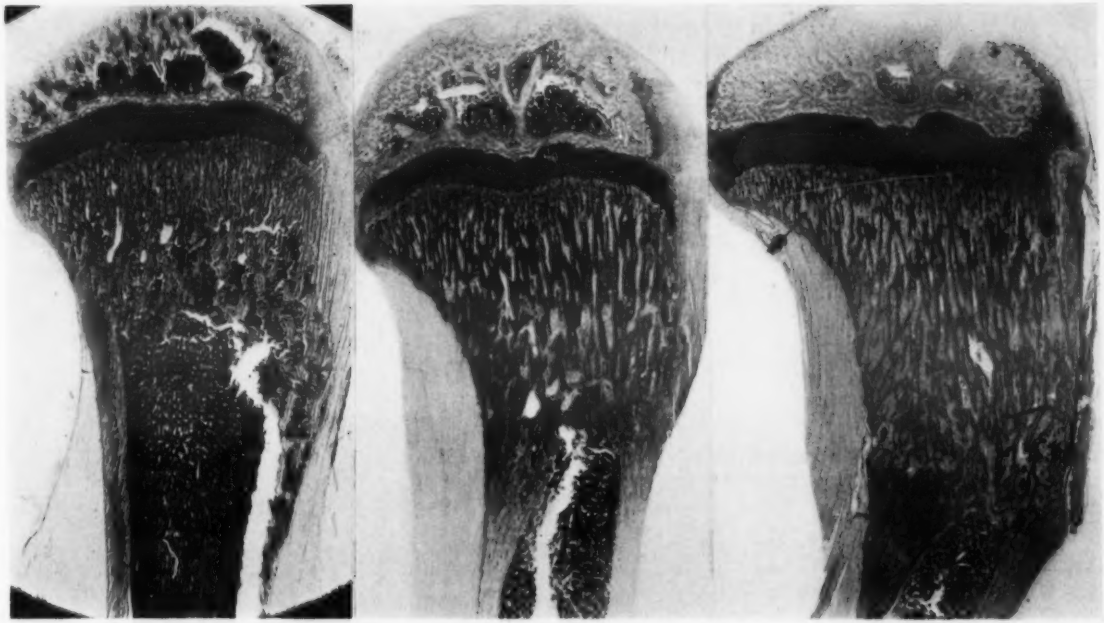
FIG. 1

TABLE II

Effect of parathormone on the serum calcium and inorganic phosphorus and on the bones of young rats maintained on the Steenbock-Bills stock diet (Ca = 0.475 and P = 0.450 gram per cent)

RAT NO.	AGE	PARATHORMONE DAILY	DURATION	SERUM Ca	SERUM INORGANIC P	AUTOPSY	X-RAY	HISTOLOGY
	days	*units*	*days*	*mgm. per cent*	*mgm. per cent*			
3057 3058 3059	18	10	14	14.2	9.9	Bones hard to cut. Increased density and number of trabeculae, extending into marrow cavity	Osteosclerosis. Dense shadow below metaphysis, extending for a distance into marrow cavity	Hyperostosis. Moderate widening of cartilage zones. Increased trabeculation and new bone formation. Osteoblastic activity increased. (See text)
3060 3061	18	None. Controls	14	12.9	6.0	Normal bones	Normal bones	Normal bones
3082 3083 3084	24	10	21	12.9	8.9	Same as 3057–9	Same as 3057–9	Same as 3057–9
3085 3086	24	None. Controls	21	11.8	5.0	Normal bones	Normal bones	Normal bones
3101 3102 3103	31	1.0	21	13.1	9.2	Slight increased density and trabeculation. Not as marked as with 10 units	Same as autopsy notes	Same as autopsy notes

3104 3105 3106	31	0.1	21	12.7	7.5	Only slightest suggestion of sclerosis. Nearly normal bones	Same as autopsy notes	Same as autopsy notes
3346 3347 3348	35	20	5	13.9	3.6	Bones very soft and break easily. Trabeculae nearly entirely disappeared	Most marked decalcification. Areas suggestive of cysts	Marked fibrosis of shafts, spongiosa and marrow
3349 3350 3351	35	None. Controls	5	—	—	Normal bones	Normal bones	Normal bones
3426 3427 3428 3429	35	20	7–14	—	—	All died 7–14 days. Calcification of organs	Rarefaction of bones	Marked rarefaction and fibrosis
3515 3516 3517 3518	26	20	4	12.3	12.7	Calcification of heart and kidneys	Rarefaction of bones	Marked rarefaction and fibrosis
3477 3480	60	20 u. per 100	21	13.1 14.0	7.1 5.3	No. 3480 metastatic calcification in heart, kidney and stomach. Bones hard. Areas of decalcification and sclerosis	Areas of decalcification and sclerosis. No. 3480 cystic area in lower part of fibula	Areas of sclerosis and fibrosis
3478 3479	60	None. Controls	21	11.3 12.0	4.5 5.1	Normal bones	Normal bones	Normal bones



3085 3084 3059

FIG. 2. No. 3085, control, stock diet. No. 3084 and no. 3059, stock diet and 10 units parathormone daily for twenty-one and fourteen days, respectively. Rat no. 3084 was twenty-four days and no. 3059 was eighteen days old when injections were begun. Note the extreme degree of trabeculation, especially in no. 3059, as contrasted with the control; the slight amount of fibrosis (arrow) between the uppermost trabeculae and also the distance between the cartilaginous zone and the marrow cavity.

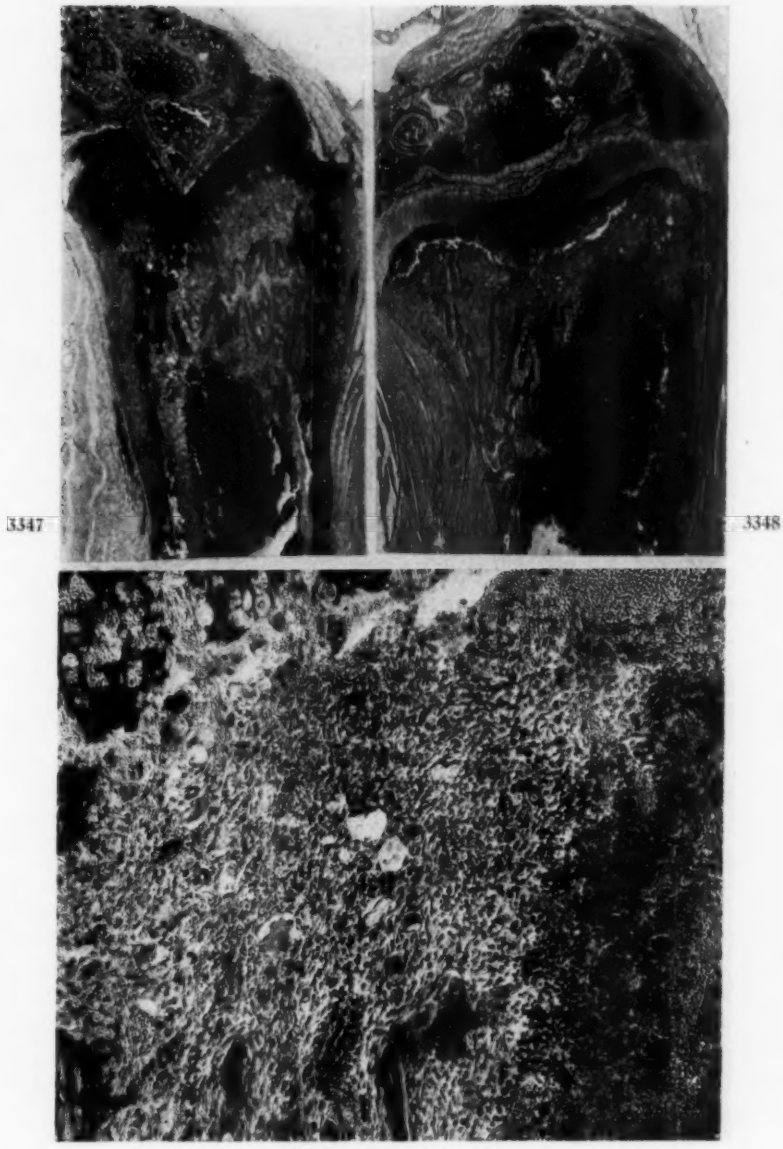


FIG. 3. Stock diet and 20 units of parathormone daily for 5 days. Note the disappearance of trabeculae and replacement with fibrous tissue. The hemorrhage and the fibrosis seen in no. 3348 are shown in higher magnification in no. 3348-A.

concentrated around areas undergoing decalcification. The diaphyses also showed evidence of extreme decalcification and fibrous replacement with few or no marrow cells remaining. Osteoid tissue was not as abundant as is commonly described in clinical chronic osteodystrophia. This was probably due to the acuteness of the destructive process; and had the process been more chronic and of longer duration, more osteoid tissue would have been present as a means of

3068 3108

FIG. 4. No. 3068, Steenbock diet with 0.1 per cent viosterol 1-D, and no. 3108, the same diet and 10 units parathormone daily for twenty-one days. Note the nearly complete absence of rickets or of trabecular proliferation in no. 3068, and the presence of a wide zone of cartilage, osteoid tissue and trabecular proliferation and no tendency to heal in the zone of preparatory cartilage in no. 3108.

support and reinforcement for the lack of rigidity occasioned by decalcification. Metastatic calcification was observed in the soft tissues —chiefly in the kidneys, heart, lungs and stomach. The skin was not examined histologically, but there was no gross evidence of calcification or of scleroderma.

2. *Steenbock rachitogenic diet.* If the contention of Taylor (18) and others that the effects of vitamin D are identical with those produced by parathormone, it would follow that parathormone should prevent

the development of rickets in the same manner as minimal doses of viosterol do. In order to prove or disprove this assertion, five groups of rats were fed the Steenbock rachitogenic diet no. 2965 for twenty-one days, during which time three groups were also injected with either 10.0, 1.0, or 0.1 units of parathormone daily; a fourth group received 0.1 per cent of viosterol 1–D in the diet, and the fifth group received no treatment at all. At the end of this period, the animals were killed and their bones were examined roentgenologically and histologically for the presence or absence of rickets. The bones of the control animals showed florid rickets and those from the rats receiving 0.1 per cent of viosterol 1–D in their diet, but no parathormone, showed no evidence of rickets (figs. 1 and 4, 3068); while those from the rats receiving 10 units of parathormone daily, instead of vitamin D, presented two distinct reactions: (1) an exaggeration of the rachitic metaphyses and (2) osteoblastic proliferation and stimulation of osteogenesis (figs. 1 and 4, 3108). The severity of the rickets was evidenced by the unusually wide metaphyses which contained much osteoid and irregular tongues of cartilage, by the absence of healing in the provisional cartilage, and by the presence of much osteoid tissue in the shafts. The stimulating effect of parathormone on the trabeculae was, in some respects, the same as that observed when the hormone was given to animals receiving the stock diet except that the trabeculae were poorly calcified and were surrounded by large amounts of osteoid tissue. The osteoblastic proliferation was quite active about the margins of the trabeculae and about the endosteum of the poorly calcified shafts.

With 1.0 unit of parathormone daily, the above effects on the bones were present only to a slight extent; and with 0.1 unit daily the bones differed little from the rachitic controls, except for a very slight increase in osteoblastic activity about the trabeculae and endosteum.

The serum inorganic phosphorus concentrations of the injected animals remained at low levels, indicating that parathormone does not behave like vitamin D in the rachitic organism; the calcium concentrations, however, were moderately elevated, especially when larger doses of parathormone were used (table III).

3. *The effect of dietary phosphorus and variations in the calcium : phosphorus ratio of the diet on parathormone activity.* From the above experiments two facts become evident: (1) that parathormone does not

TABLE III

Effect of parathormone on the prevention of rickets. Steenbock diet no. 2965 (Ca = 1.240; P = 0.250 gram per cent)

RAT NO.	AGE	PARATHORMONE DAILY	DURATION	SERUM Ca	SERUM INORGANIC P	AUTOPSY	X-RAY	HISTOLOGY
	days	*units*	*days*	*mgm. per cent*	*mgm. per cent*			
3107 3108 3109	29	10	21	14.1	3.2	Bones soft. Wide metaphysis. Slight sclerosis in area adjacent to rachitic metaphysis	Rickets marked. Same as Autopsy	Rickets marked. No healing in provisional zone. Moderate increase in number of trabeculae which are poorly calcified. Increased osteoblastic activity
3110 3111 3112	29	1.0	21	12.9	3.3	Marked rickets. Osteoporosis	Same as autopsy	Same as autopsy. Very slight increase in number of trabeculae which are poorly calcified
3113 3114 3115	29	0.1	21	12.8	3.3	Marked rickets and osteoporosis	Same as autopsy	Slight increase in osteoblastic activity
3066 3067 3068 3069	29	Viosterol 1-D 0.1%. No parathormone	21	10.5	4.2	Complete protection	No rickets	No rickets. Slight osteoporosis

prevent rickets and hence does not behave like vitamin D in that respect; and (2) that the hyperostosis induced by parathormone injections occurs more intensely when the dietary phosphorus is optimal, and to a much lesser degree when the phosphorus intake is low. The latter fact suggested that, as in normal ossification, an abundance of lime salt and a proper calcium : phosphorus ratio are requisites for the pro-

3117 3000

FIG. 5. No. 3107, Steenbock diet with added Na_2HPO_4 (calcium:phosphorus = 1:0.05); no. 3000, same with 10 units parathormone daily. Note the proliferation of the cartilaginous zone and the hyperostosis in the treated as compared with the control.

duction of the sclerotic changes in the trabeculae by parathormone. Consequently, experiments were performed in which injected and control animals were fed the Steenbock rachitogenic diets in which the calcium : phosphorus ratios were changed either by adding Na_2HPO_4 or reducing the percentage of $CaCO_3$. In the first series enough Na_2HPO_4 was added to the diet to make the phosphorus content about

TABLE IV

Effect of parathormone on the bones of young rats fed a "border-line" rachitogenic diet. (Steenbock diet no. 2965 + Na_2HPO_4; Ca = 1.240, P = 0.620, or Ca:P = 2:1)

RAT NO.	AGE	PARATHORMONE DAILY	DURATION	SERUM Ca	SERUM INORGANIC P	AUTOPSY	X-RAY	HISTOLOGY
Experiment A. Alkaline diet								
	days	*units*	*days*	*mgm. per cent*	*mgm. per cent*			
2998 2999 3000 3001	30	10	27	14.7	6.5	Cartilaginous zone moderately widened. Moderate hyperostosis of submetaphyseal zone. Bones hard to cut	Same as autopsy	Cartilage disc much wider than normal. In some it is as wide as in moderate degrees of rickets. Osteoblastic activity pronounced. Proliferative trabeculae well calcified but not as pronounced as on stock diet
3036 3037 3038	19	10	21	14.0	6.0	Same as nos. 2998–3001	Same as nos. 2998–3001	Same as nos. 2998–3001
3040 3041 3042	19	None	21	—	—	Normal bones	Normal bones	Normal bones
3116 3117 3118	27	None	21	11.5	5.2	Normal bones	No rickets	Normal bones

3119 3120 3121	27	10	21	12.1	6.9	Same as nos. 2998–3001	Same as nos. 2998–3001	Same as Nos. 2998–3001
3122 3123 3124	27	1.0	21	12.0	5.6	Bones fairly hard to cut. Slight hyperostosis	Same as autopsy	Slight widening of cartilage zone. Slight hyperostosis. Trabeculae well calcified
3125 3126 3127	27	0.1	21	12.5	6.5	Normal bones	Normal bones	Normal bones. Possibly trabeculation somewhat denser than controls
Experiment B. Diet rendered acid by substituting equimolar $CaCl_2$ for 3.0 grams per cent $CaCO_3$								
3052 3053 3054	24	10	29	13.4	Too faint to read	Marked rickets. Bones soft	Marked rickets	Rickets and osteoporosis. Increased osteoblastic activity about trabeculae
3055 3056	24	None. Control	29	12.8	Too faint to read	Moderate rickets	Moderate rickets	Moderate rickets. Not as severe as parathormone treated animals
Experiment C. Diet rendered moderately acid by substituting equimolar $CaCl_2$ for 1.5 grams per cent $CaCO_3$								
3087 3088 3089 3090	22	10	21	13.3	4.0	Slight widening of cartilaginous zone. Trabeculae hyperostotic	Moderate widening of cartilage zone. Hyperostosis	Cartilage disc wide; extensive increase in trabeculations which are only moderately well calcified
3091 3092 3093	22	None. Control	21	11.9	3.6	Trabeculae normal	Very slight widening of cartilage. Trabeculae normal	Cartilage zone nearly normal. Osteoporosis (?)

0.620 gram per cent and the calcium : phosphorus ratio 2:1. Park, Mendel and Jackson (20) found that this diet induces rickets in rats but rarely, and hence it may be considered a "border-line" rachitogenic diet. The bones of the animals injected with 10 units of parathormone daily showed changes in the trabeculae similar to those seen when the stock diet was fed, but to a much lesser degree. The trabeculae did not extend nearly as far into the marrow cavity nor were they as compact; osteoid was present in moderate amounts about the trabeculae and the shafts; and the degree of calcification and the osteoblastic activity were not as pronounced. The cartilaginous zones, however, were much wider and nearly as wide as that observed in moderately severe rickets (figs. 1 and 5, 3000). With 1.0 unit of parathormone, the osseous changes were less marked and with 0.1 unit daily the changes were slight and the bones resembled nearly those from the control animals (table IV).

Apparently the slight stimulating effects of parathormone to cartilage proliferation observed in animals fed the stock diet was intensified in the animals fed a diet in which the calcium content was twice that of the phosphorus. Also, the enlarged trabeculae, which were poorly calcified when the dietary calcium was high and the phosphorus low, became impregnated with lime salts when the phosphorus intake was doubled.

The experiment was repeated with diets which were similar but made more acid by replacing equimolar amounts of $CaCl_2$ for $CaCO_3$ (table IV). The results, when 10 units of parathormone were given daily, were similar to those obtained with the alkaline diet except that the bones of the treated as well as of the control animals appeared somewhat more osteoporotic, and calcification in the hyperostotic trabeculae was less marked. The rachitic metaphyses were rather wide, indicating a mild or moderate degree of rickets. In the treated animals, in spite of the inferiority of calcification and the presence of osteoid, the parathormone effect was evident by the increased trabeculation, and the presence of large cells and many osteoblasts.

In the next series, the animals were also fed the Steenbock diet containing a calcium : phosphorus ratio of 1.8:1 but, instead of altering the ratio by adding Na_2HPO_4 to the diet, as was done in the previous experiment, the calcium intake was reduced and the dietary phos-

TABLE V

Effect of parathormone on the bones of rats fed the Steenbock diet no. 2965 in which the Ca:P ratio was reduced to 1.8:1 by using 1.0 gram instead of 3.0 grams per cent $CaCO_3$

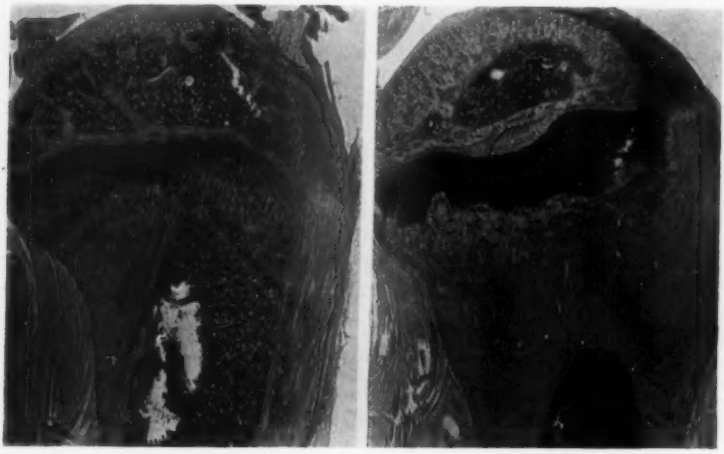
RAT NO.	AGE	PARATHORMONE DAILY	DURATION	SERUM Ca	SERUM INORGANIC P	AUTOPSY	X-RAY	HISTOLOGY
	days	*units*	*days*	*mgm. per cent*	*mgm. per cent*			
3026 3027 3028 3029	30	10	31	13.5	3.5	Marked rickets	Marked rickets	Rickets. Very little calcification in proliferative trabeculae
3043 3044 3045 3046	30	None. Control	31	12.5	3.9	Mild to moderate rickets	Mild to moderate rickets	Mild to moderate rickets

TABLE VI

Effect of parathormone on the bones of rats fed the Steenbock diet no. 2965 in which the calcium and phosphorus were kept at approximately 0.250 gram per cent (Ca·P = 1:1)

RAT NO.	AGE	PARATHORMONE DAILY	DURATION	SERUM Ca	SERUM INORGANIC P	AUTOPSY	X-RAY	HISTOLOGY
	days	*units*	*days*	*mgm. per cent*	*mgm. per cent*			
3337 3338 3339	29	None. Controls	21	7.7	5.0	No rickets; moderate osteoporosis	Same as autopsy	Same as autopsy; cartilage disc very narrow
3340 3341 3342	29	10	21	5.3	14.3	Moderate rickets, and sclerosis	Moderate rickets and sclerosis	Moderate rickets. Cartilage disc quite wide. Moderate sclerosis

phorus was left at its former low level. This was accomplished by using only 1.0 gram per cent of $CaCO_3$ instead of 3.0 grams of the usual Steenbock rachitogenic diet. Ten units of parathormone were injected daily for thirty-one days (table V). The bones from the control animals showed mild or very moderate rickets, while those from the injected animals showed more advanced stages of the disease. It differed from ordinary rickets in that the trabeculae were greater in number and the osteoblastic activity was much more pronounced.



3339 3342

FIG. 6. No. 3339, Steenbock diet with only 0.5 gram per cent $CaCO_3$ (control). Note absence of rickets or hyperostosis. No. 3342, same diet with 10 units parathormone daily. Note the proliferation of the cartilaginous zone and the moderate hyperostosis in the subcartilaginous zone.

In the third series, the $CaCO_3$ of the above diet was reduced to 0.5 gram per cent so that the calcium and phosphorus were both at about 0.250 gram per cent and the calcium:phosphorus ratio about 1:1 (table VI). In contrast with the last mentioned diet which produces a mild or moderate rickets, this low calcium and low phosphorus diet produced poorly calcified bones but *not rickets*, *i.e.*, the cartilaginous zone and the metaphysis remained very narrow (figs. 1 and 6, 3339). However, the bones of animals fed this diet, and injected with 10 units

TABLE VII

Effect of parathormone on the bones of rats fed a diet in which the calcium was reduced to only 0.100 gram per cent and the phosphorus kept at approximate level of 0.280 gram per cent (Ca:P = 0.35:1)

RAT NO.	AGE	PARATHORMONE DAILY	DURATION	SERUM Ca	SERUM INORGANIC P	AUTOPSY	X-RAY	HISTOLOGY
	days	*units*	*days*	*mgm. per cent*	*mgm. per cent*			
3128 3129 3130 3131	27	None. Control	21	11.2	9.3	Slight osteoporosis	Slight osteoporosis	Slight osteoporosis
3132 3133 3134 3135	27	10	21	11.8	6.3	Moderate widening of cartilaginous zone. Moderate hyperostosis	Same as autopsy	Moderate to mild rickets. Moderate increase in trabeculae
3161 3162 3163 3164	30	1.0	21	12.5	10.0	Very slight hyperostosis	Same as autopsy	Same as autopsy
3165 3166 3167 3168	30	0.1	21	11.7	10.2	Cartilage zone narrow; same as controls	Same as autopsy	Same as autopsy

TABLE VIII

Effect of parathormone on the bones of rats fed the stock diet with added Na_2HPO_4, Ca = 0.475, P = 1.100 gram per cent (Ca:P = 1.2:3)

RAT NO.	AGE	PARATHORMONE DAILY	DURATION	SERUM Ca	SERUM INORGANIC P	AUTOPSY	X-RAY	HISTOLOGY
	days	*units*	*days*	*mgm. per cent*	*mgm. per cent*			
3570 3571 3572	25	10	15	13.4	10.9	Moderate sclerosis and decalcification	Areas of decalcification and sclerosis	Areas of fibrosis and sclerosis. Fibrosis more pronounced than on stock diet and 10 units parathormone
3573 3574 3575	25	1.0	15	13.7	10.0	Slight sclerosis	Slight sclerosis	Slight sclerosis
3576 3577 3578	25	0.1	15	13.6	10.2	Nearly normal bones	Nearly normal bones	Same as autopsy
3579 3580 3571	25	None. Control	15	12.9	10.5	Normal bones	Normal bones	Nearly normal bones

of parathormone daily, showed (1) proliferation of the cartilaginous zone and formation of osteoid tissue to a degree seen in moderately severe rickets, and (2) a moderate degree of trabecular sclerosis (figs. 1 and 6, 3342).

In the fourth series, the phosphorus of the diet was kept low (0.280 gram per cent) and the calcium was further reduced to about 0.100 gram per cent, so that the calcium:phosphorus ratio was about 0.35:1 (table VII). The cartilaginous zones of the bones of the control animals on this diet were extremely narrow and the shafts were markedly osteoporotic, while the bones of the animals receiving 10 units of parathormone daily for twenty-one days showed moderate widening of the cartilage discs to a degree seen in moderate rickets, and also moderate sclerotic changes and osteoblastic proliferation in and about the trabeculae. These changes were less marked in the group receiving 1.0 unit of parathormone and nearly absent in those treated with 0.1 unit daily. Apparently, in this series as in the third series, parathormone in doses of 10 units daily stimulated cartilage proliferation and osteoblastic activity about the osseous trabeculae.

In the fifth and last series the calcium of the diet was kept at an optimal level while the phosphorus was increased to 1.100 grams per cent by the addition of 2.75 grams per cent Na_2HPO_4 (anhydrous) to the Steenbock-Bills stock diet. Parathormone, in doses of 10, 1.0 and 0.1 units, was injected daily for 15 days (table VIII). In contrast to the predominance of hyperostosis observed in the bones of the animals fed the stock diet and injected with 10 units of parathormone daily. the bones of the animals in the high phosphorus group, so treated, showed some hyperostosis but a greater tendency to decalcification (figure 1, 3571). The bones of those receiving 1.0 unit of parathormone daily showed slight hyperostosis and of those injected with 0.1 unit daily differed but little from the bones of the control animals receiving no treatment.

The tendency for decalcification in the above group treated with 10 units of parathormone daily, as contrasted with the hyperostosis seen in the groups receiving the stock diet, may be due to the fact that the excess of phosphorus over calcium in the diet in itself favors loss of calcium from the bones, and parathormone merely intensifies this process, thus leading to decalcification.

4. *Effect of Parathormone on the Bones of Suckling Rats.* Recently Selye (14) reported that the injection of large doses of parathormone into ten-day-old suckling rats produced, within six days, a histologic picture resembling rickets. We have made similar experiments in order to ascertain the effects of parathormone on the centers of ossification in the epiphyses. In the first experiments, one half of a litter of eight newly born rats was injected with 10 units of parathormone daily from the first day of life until the animals were seventeen days old, while the other half of the litter served as a control. The control animals nursed and grew normally, but the treated animals nursed poorly and two died shortly after injections were begun. Those surviving presented moderate rarefaction of the bones but *not rickets*.

In the next experiment, another litter of eight siblings, ten days old, and averaging in weight 15 grams each, were divided into two equal groups. One group served as a control while the other was injected with 5 units of parathormone per rat daily for ten days, at which time the experimental as well as the control animals were killed and the presence or absence of rickets was ascertained. Radiographically and histologically the bones of the control animals showed epiphyseal and cartilaginous developments which were normal for that age; while those from the injected animals showed marked fibrosis and replacement of the marrow and moderate proliferation of cartilage in the metaphyses and in the epiphyses, so that calcification in the epiphyseal centers was delayed. In contrast to the predominance of osteoid in the shaft and especially the metaphysis of rachitic bones, the metaphyses and the shafts of the bones from parathormone treated animals were composed, nearly entirely, of fibrous tissue (fig. 7). Evidently the bone lesions produced in these animals by parathormone are *not those of rickets*.

Another attempt to produce rickets in ten-day-old rats by the injection of 10 units of parathormone daily was met with complete failure, as most of the animals died within a few days after the injections were begun. Even those receiving only 5 units daily nursed poorly and gained very little weight.

The rats treated with 5 units of parathormone daily developed peculiar lesions of the skin, similar to those described by Selye (21) and which he believes to be scleroderma. These lesions became apparent

3375–A

3375–B

3375–C

FIG. 7. No. 3375-A. Control rat, 20 days old, mother on stock diet.

No. 3375-B. Litter mate, same age. Treated with 5 units parathormone daily for ten days. Note the delay in calcification in the epiphyseal centers, the moderate proliferation of the cartilaginous zone and the extreme fibrous replacement.

No. 3375-C. Shows the decalcified area as being composed of *fibrous* tissue as seen in osteodystrophia rather than of *osteoid* seen in typical rickets.

as early as the second day after the injection and became progressively worse as the injections continued. First, the hair about the nape of the neck and shoulders became fuzzy and sparse, then the skin over the back and extremities became thick and wrinkled. In the succeeding few days the animals appeared as if the skins were too large for their bodies, as folds were noted about the legs and neck. The skins appeared thicker and harder and the animals kept scratching themselves nearly continuously. At autopsy, the skin stripped from the skeleton with ease and the subcutaneous fat was of a much darker yellow color than in the control litter mates, which were kept in the same cage but had none of these lesions. Grossly, the skins were inelastic and leathery and about four to five times the thickness of normal skin. The results of their histological examinations will be reported elsewhere.

5. *Effect of Diet and of Parathormone on Growth and Health.* In growth and well-being the animals receiving parathormone differed but little from their controls, with the exception of those fed the stock diet and injected with 10 or 20 units of parathormone daily. Those injected with 10 units, although they made poorer gains in weight than their corresponding controls, yet they did not show general ill effects, except in one instance, namely, when one of two larger rats was injected for twenty-one days with 10 units of parathormone per 50 grams of body-weight. In this rat parathormone produced, aside from loss of weight, calcification in the myocardium, the kidneys, and the wall of the stomach. Those injected with 20 units of parathormone for twenty-one days declined rapidly in weight and health. Some died within five days while others survived as long as fourteen days. Autopsy of those which died or were killed revealed extensive gross calcic deposits in the kidneys, myocardium, stomach and blood vessels, similar to those described by Heuper (22) in dogs poisoned with parathormone. Apparently, the rapid removal of lime salts from the bones, brought about by large doses of parathormone, and the resulting hypercalcemia or hyperphosphatemia predisposed the animals to abnormal calcification in the soft tissue.

The growth in the groups of animals injected with 10 units daily and fed rations other than the stock diet differed little from that of their corresponding controls. This may be explained on the ground that these rations, which were based on the Steenbock rachitogenic diet,

are inferior to the stock diet in many respects and induce inferior growth equally in injected and in control animals.

All the animals injected with the larger doses of parathormone showed symptoms of vasodilation for a short time after each injection. The ears, legs and mucous membranes assumed a pink coloration and the ears and extremities exhibited fine tremors. These symptoms, however, were only temporary and not alarming and the animals appeared normal within an hour or two after injection. The pathological physiology of this phenomenon was not investigated, but it may be due to either vasodilation alone or to a combination of vasodilation and polycythemia, since both phenomena are met with, not infrequently, in dogs treated with large doses of parathormone (23).

6. *Effect of Diet and Parathormone on the Concentrations of Serum Calcium and Inorganic Phosphorus.* The concentrations of serum calcium and inorganic phosphorus on the various diets and doses of parathormone are shown in tables II to VIII. It is seen that, depending upon the diet and dose of parathormone, moderate to high levels of serum calcium may be obtained in the rat and hence the rat is not entirely immune to the effects of parathormone, as claimed by some. It must be admitted, however, that the doses employed are quite out of proportion to those used in dogs or human beings to cause an equal degree of hypercalcemia.

On the stock diet the levels of serum calcium and inorganic phosphorus were somewhat higher in the injected animals than in the controls—the phosphorus was increased more than the calcium. In one of the groups injected with 20 units of parathormone daily, the serum inorganic phosphorus levels were extremely high, but the calcium concentrations were not materially elevated. Similar high phosphorus levels in the serum were noted by Collip (4) in dogs injected with large doses of parathormone and the hyperphosphatemia probably indicates a severe overdosage.

On the Steenbock rachitogenic diets, the serum inorganic phosphorus levels in the injected animals remained low, while the calcium concentrations were higher than in the corresponding parathormone-treated groups fed the stock diet. The failure of the serum inorganic phosphorus to increase in the injected animals as compared with that in animals treated with viosterol indicates, again, the dissimilarity of parathormone and vitamin D.

On the Steenbock diet to which Na_2HPO_4 was added or from which a portion of the $CaCO_3$ was omitted so that the calcium:phosphorus ratio of the diet was about 2:1, parathormone, even in large doses, produced only moderate hypercalcemia and but little or no effect on the serum inorganic phosphorus. The absence of the parathormone effect on the serum inorganic phosphorus on these diets and also on the rachitogenic diet may be attributed, in the latter cases, to the paucity of dietary phosphorus and, in the former, to an excess of dietary calcium over phosphorus as compared with that contained in the stock diet. When the diet to which Na_2HPO_4 was added was rendered acid in reaction, parathormone produced only a slight elevation in the serum calcium but the inorganic phosphorus was not elevated at all; and in two instances it was so low that it was impossible to determine it colorimetrically. The low serum inorganic phosphorus concentrations in these instances may be due to the reciprocal hyperchloremia produced by the large intake of chlorides as $CaCl_2$.

The serum inorganic phosphorus of the groups receiving the stock diet and Na_2HPO_4 and injected with parathormone differed little from that of the uninjected controls. It was high in all the groups as a result of the high phosphorus ingestion. The serum calcium levels were only slightly higher in the injected groups.

In the animals receiving a low calcium and low phosphorus diet, (Calcium = 0.250 and Phosphorus = 0.250 milligrams per cent), parathormone produced an effect which was not anticipated. In the control group the serum calcium and inorganic phosphorus were 7.7 and 5.0 milligrams per cent, respectively; while in the group injected with 10 units of parathormone daily the serum calcium was only 5.3 and the inorganic phosphorus 14.3 milligrams per cent, respectively. In spite of the low serum calcium, frank tetany was not noted, although it is possible that mild tetany had been overlooked. A similar hypocalcemia or absence of hypercalcemia in chronic hyperparathyroidism in dogs was described by Bodansky and Jaffe (24). They suggested, tentatively, that the hypocalcemia or the absence of hypercalcemia in their dogs fed low calcium diets and treated with parathormone may be due to a depletion of tissue calcium and to a too rapid excretion of calcium in the urine. But, since this was not proved experimentally, it is equally possible that, in the presence of a low calcium intake, parathor-

mone caused a diversion of calcium from the serum to the bones. This is evidenced by the moderate sclerosis of the bones of our treated animals and also by the observations of Bülbring (15) that parathormone caused retention of calcium even when the intake of this element was low. The low serum calcium may also have been due to the excessively high concentration of inorganic phosphorus.

The recognition of the relation of diet to the response of parathormone is important not only theoretically but also clinically, as already pointed out by Bodansky and Jaffe. Quite frequently the diagnoses of hyperfunction or hypofunction of the parathyroids are made on one or more determinations of serum calcium without regard to previous intake of calcium and phosphorus; and there may be instances where a diagnosis of hypoparathyroidism was made and parathormone therapy instituted because of a relatively low serum calcium level, although, clinically, the patients may have had symptoms of hyperfunction of the parathyroids. For this reason it would seem that in clinical cases the calcium and phosphorus intake should be controlled for at least a few days prior to determining the serum calcium as an aid in the diagnosis of parathyroid disease.

In general, the highest values for serum calcium were obtained in the groups receiving the high calcium-low phosphorus diets and large doses of parathormone and the lowest figures in the groups receiving diets deficient in calcium. These findings are in accord with those of Bülbring.

7. *Effect of variations in dietary calcium and phosphorus and of dosage of parathormone on the ash and calcium and phosphorus percentages of the bones.* In the study of the effects of antirachitic agents in favoring calcification, the determination of the ash percentage of the dry extracted bones gives a rough indication of the degree of calcification, as compared with that of rachitic bones. The ratio of ash to organic matter (A/R) as an index to calcification is thus frequently used in the biological assay of vitamin D. It has also been shown by Bethke (25) and others that the addition of vitamin D to otherwise optimal diets also increases the percentages of ash and lime salts in the bones, *i.e.*, it either increases the amount of lime salts without at the same time increasing the organic material or that the organic matter decreases as lime salt deposition increases. While these changes may take place

in the bones from rachitic animals treated with vitamin D or from animals fed apparently optimal diets and moderately large doses of vitamin D, the mechanism of increased lime deposition in the bones of the parathormone-treated animals fed the stock diet seems to be quite different. Figure 8 shows that the percentages of ash and calcium and phosphorus of the bones of the parathormone-treated animals presenting osteosclerosis are actually somewhat lower than in the untreated controls, yet roentgenologically and histologically there is definite evidence of increased calcification. Evidently parathormone primarily stimulates osteoblastic activity and increased trabeculation, and the calcification which follows does not exceed the formation of organic matrix, so that the absolute increase in calcification is not reflected in the percentage of lime salts nor in the ratio of ash to organic matter. However, the definite hyperostosis was ascertained in another way, *i.e.*, by determining the relative increase of the ratio of weight of each extracted dry femur to the body weight of the corresponding animal in this series. The ratio in the control groups fed the stock diet averages 0.00138 and is in accord with figures (0.00135) calculated from Hammet (26) for normal rats of similar ages. The ratios in the groups receiving 0.1, 1.0, and 10.0 units of parathormone daily were 0.00147, 0.00151, and 0.00160, respectively, indicating increments of bone weight proportional to the dose of parathormone.

The percentages of ash and lime salts of the bones of animals fed the stock diet and treated with 20 units of parathormone daily were much below the normal values (fig. 8) and approached figures usually seen in rickets or extreme osseous decalcification due to other causes. The reduction of the ash and increase in the organic matter were due largely to replacement of calcified material by uncalcified fibrous tissue.

The ash and lime salt analyses of the rachitic rats also gave but little indication as to the state of calcification brought about by the various doses of parathormone. Since in rachitic bones there is already a preponderance of organic material over ash, and since the percentages of ash and calcium vary with the degree of rickets in each individual animal, not much reliance can be placed on slight changes. However, the average for the groups showed slight but definite increases commensurate with dosage of parathormone (up to 10 units) and is probably due to the slight increase of calcification in the proliferating trabeculae.

The highest increments, however, never equalled even that obtained by feeding the same diet and only *minimal* prophylactic doses of viosterol. These analyses taken by themselves, without resorting

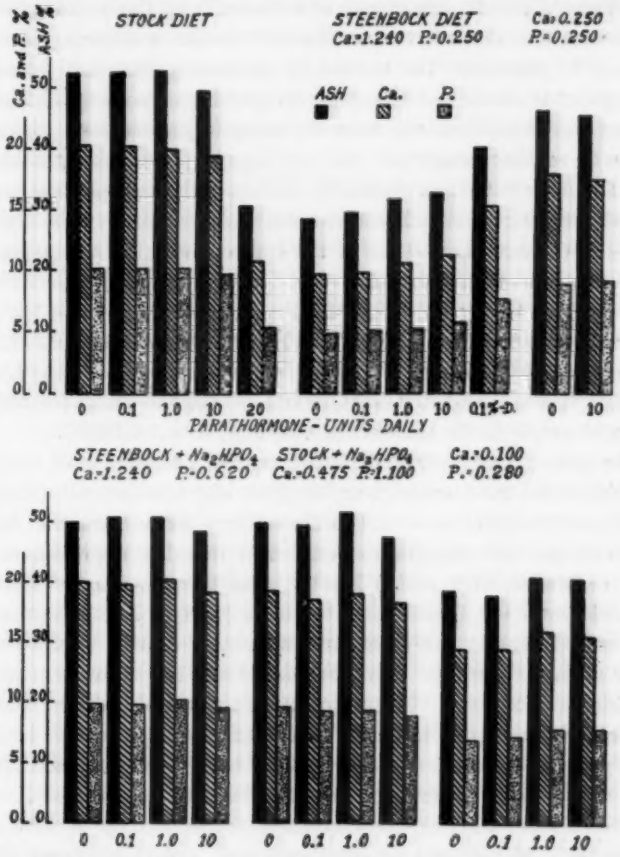


FIG. 8. The ash and calcium and phosphorus percentages of the dry extracted femurs (average of three rats) of the different groups. 0.1 per cent -D = 0.1 per cent of viosterol 1-D in the diet.

to histological or roentgenological examinations of the bones, may be very misleading in that these slight effects of parathormone might be

compared to those of small doses of viosterol. Histologically, however, it was evident that parathormone did not prevent rickets, in spite of the seemingly slight increase in bone ash. As with the stock diet, the effects of parathormone and of vitamin D on the histology of the bones were also dissimilar when the rachitic diet was used. Here too vitamin D prevented the rickets by increasing lime salt deposition throughout the bone and especially the provisional zone of calcification so that the metaphysis was narrow; whereas *parathormone had no effect on the rachitic metaphysis*—the cartilage layers remaining unusually wide and distributed in a disorderly fashion in the metaphyseal osteoid. The slightly increased ash content was due, primarily, to a slight increase in the number and size of the trabeculae in the spongiosa.

The ratio of femur weight to body weight in the groups receiving rachitic diets was quite different from those obtained with the stock diet although an upward trend was also noted in the treated animals. In the parathormone rats, the figures were as follows: 0.1 unit, 0.00119; 1.0 unit, 0.00131; and 10.0 units, 0.00147. For the group treated with minimal prophylactic amounts of viosterol, it was 0.00157.

The percentage of ash and of calcium and phosphorus of the bones of animals fed the Steenbock rachitogenic diet fortified with Na_2HPO_4 (Calcium:Phosphorus = 2:1) differed very little from the normal. The calcium and phosphorus content of this diet approximates diet no. 4 of Bethke, Kick, and Wilder (25), and the percentage of ash of our control group (49.8) is similar to theirs (49.4). The percentages of ash and of lime in the parathormone-treated animal were about the same as in the controls—the differences being too slight to attach any significance to them. The ratio of femur to body weight was only moderately increased in the 1.0 and 10 units group, but not in the 0.1 unit group. We have not analyzed the bones of animals fed this diet and viosterol, but from the analyses of Bethke and others it is seen that on similar diets viosterol increased the percentage of bone ash by more than 16 per cent as compared with the insignificant changes noted in the bones of our parathormone-treated animals. This again demonstrates the dissimilarity between the physiologic behavior of vitamin D and parathormone.

The ash and lime percentages of the bones of the animals fed low calcium-low phosphorus diets (Calcium:Phosphorus = 1.1 or 0.35:1)

and treated with parathormone differed little from those of the bones of the untreated control animals—in spite of moderate hyperostosis. However, the ratio of femur to body weight showed definite increases over that of the controls. In the groups fed the stock diet with Na_2HPO_4, a slight increase in the ash and lime percentage of the femurs was noted in the 1.0 unit group and a moderate decrease in the group receiving 10 units of parathormone daily. The decrease was apparently due to the presence of fibrosis and areas of decalcification.

DISCUSSION

The results on the effects of parathormone in the rat present several interesting points. First, that the rat is not entirely immune to the effects of parathormone, with respect to its ability to raise the level of calcium in the serum. Our results indicate that moderate or even dangerous hypercalcemias and hyperphosphotemias may be obtained in the rat by this hormone, but that the levels of the serum calcium and inorganic phosphorus depend, in part, on the calcium and phosphorus of the diet and the dosage of parathormone. It must be admitted, however, that a definite species resistance does exist in the rat with respect to its response to *bovine* parathormone, since the doses employed in order to obtain effects in rats are out of proportion to the amounts necessary to produce a similar hypercalcemia in other species of animals. Thus, the daily injection of 10 units of parathormone into a 50-gram rat is equivalent to a daily dose of 12,000 units of the hormone for a 60-kilo man; and even the smallest dosage employed by us (0.1 unit daily), which produced little or no effect on the serum calcium levels nor on the osseous trabeculae, amounts to a highly toxic daily dose of 120 units for the average man. Whether the tolerance of the rat to usual amounts of parathormone is a function of size and surface area or merely a species resistance cannot be said with certainty, since it is not definitely known how rats would respond to extracts of the parathyroids of the same species. It is quite possible that both factors are operative. That the dose of parathormone *may* bear some relation to surface area is suggested by the experiments of Süssman (27) and of Learner (28). They have found that mice are resistant to the effects of 10 to 20 units of parathormone daily, or an amount equal to a daily dose of 20,000 to 40,000 units in human beings. Apparently, the mouse,

because of its greater surface area per unit of body weight, can tolerate doses of parathormone which produce hypercalcemia and fatal terminations even in the rat. Our own experiments also suggest that there might be a relationship between body size and dosage of parathormone per unit of body weight. Thus, whereas our younger animals tolerated 10 units per rat or about 20 units of parathormone daily per 100 to 120 grams of body weight, our older animals weighing 120 to 150 grams were less tolerant to a similar dose per unit of body weight as is evidenced in the case of rats nos. 3477 and 3480 (table II). These rats were injected daily, for twenty-one days, with 20 units of parathormone for 100 grams of body weight. In contrast to the predominance of hyperostosis and absence of metastatic calcification in younger animals injected with a similar amount per 100 grams of body weight, the older animals reacted as if a much larger amount of parathormone had been injected, *i.e.*, the bones showed well marked decalcification, fibrosis, and cyst formation as well as some hyperostosis. One of the larger animals also showed marked calcification in the soft tissues. Possibly, this may explain the occurrence of areas of degeneration in Selye's older rats and the presence of both hyperostosis and fibrosis in some of Johnson's animals, since some of the latter's rats were twelve weeks old and were probably larger than even our sixty-day-old rats. The lack of the response of rabbits (4) to amounts of parathormone capable of producing definite hypercalcemias in adult dogs and men, may also be explained on the ground that the dose of the hormone varies with the size of the animal and hence with its surface area per unit of body weight.

The second point of interest is the effect of parathormone on the bones. From all accumulative clinical and experimental studies it is assumed that the primary function of the parathyroids is to regulate the level of calcium in the blood and thereby facilitate ossification and prevent tetany. *Hypofunction* of these glands usually leads to hypocalcemia and tetany, while *hyperfunction* is associated with hypercalcemia and decalcification of the bones, termed clinically osteitis, or osteodystrophia, fibrosa. Experimentally, the latter syndrome has been produced in the dog and guinea-pig by the injection of parathormone. In the spontaneous, as in the experimentally produced osseous lesions, the osteoclastic activity is accelerated, decalcification

takes place and the decalcified areas become cystic or are replaced by fibrous tissue. In our experiments with young rats fed an optimal stock diet, such extensive hypostotic changes occurred only when more than 10 units of parathormone were administered daily, but with smaller amounts the lesions in the bones were predominantly hyperostotic or osteosclerotic—the tendency to hyperostosis diminishing with decrease in dosage. Histologically, these hyperostotic lesions differ from osteitis fibrosa in that there is little or no evidence of decalcification and fibrosis, but rather a stimulation to osteoblastic activity about the trabeculae, the consequent calcification of these trabeculae, and a persistence and continuation of the old and the newly calcified ones. In most instances, there was also evidence of proliferation of the cartilaginous zone so that its number of layers approached that seen in moderate or mild rickets.

On diets low in phosphorus, parathormone produced proliferation of cartilage as well as stimulation of osteoblastic activity about the trabeculae and endosteum. The calcification, however, was deficient and the newly formed bone consisted largely of osteoid tissue.

The divergency of effects on the bones of the rat obtained with different dosages of parathormone may explain, in part, the contradictory results of the various investigators. Thus, in the hands of Bauer, Aub and Albright (6), 8 units of parathormone—a dose comparable to our daily dose of 10 units, produced osteosclerosis in growing rats, while 20 units daily employed by Waltner (11) produced decalcification in the same species within ten to eighteen days. Johnson's (12) "very uniform" results, if carefully evaluated, actually prove that the osseous lesions depend on the dosage of parathormone. His histologic sections and roentgenograms show that two distinct osseous lesions are present in the bones of the same animals. These diverse lesions are due, in all probability, to variations in the daily dosage of parathormone and the age and size of the animals. Hyperostosis apparently developed during the periods when no more than 10 units of parathormone were injected daily and osteitis fibrosa when the dose was increased beyond this amount—in many instances, 20 to 40 units daily. A comparison of the roentgenogram of one of his rats (no. 23), so treated, with those of our animals shows clearly that hyperostosis is present in both. Also, his figure 10, illustrating histologic findings in rat no. 1 treated with 10

units of parathormone daily, appears to be more like osteosclerosis rather than the osteitis fibrosa shown in his figure 11 of rat B injected with 40 units daily.

In Selye's experiments, the dosage of parathormone is given in but very few instances and hence comparison of results with those of others is not possible. In the one instance, where dosages are mentioned, namely, 20 units daily to a 60-gram rat, the results are not in accord with those found by Johnson (12), Waltner (11), Hoff and Homann (29), and by us.

As already pointed out by Thompson and Collip (23), the results of Lambie, Kermack and Harvey (10) are difficult to interpret. In their report, preliminary in nature, they state that 10 units of parathormone administered daily to two rats for twenty-one days produced a lessened ash content but no change in the calcium percentage, *i.e.*, a greater calcium percentage in the ash. But since the percentage of calcium in the ash is more or less constant (about 38 per cent), regardless of the actual ash content, as may be seen from our own analyses and also from those of Chick, Kerenchevsky and Roscoe (30), it is difficult to reconcile the low ash and the high calcium percentages.

Burns (31) injected normal growing rats with increasing doses of parathormone varying from 2 to 10 units daily and found no change in the ash and lime salt content of their bones as compared with the bones of normal controls. But, since roentgenological and histological examinations of the bones were not made, the negative findings are not at all surprising. Our own findings, if based entirely on analyses of the ash and lime content of the bones, without histological examination, would lead us to the same conclusion, namely, an absence of parathormone effect in the rat. However, the examination of the bones grossly, histologically, and roentgenologically and also the determination of the ratio of dry extracted femur weight to body weight demonstrate a definite parathormone effect on the bones of the rat treated with doses up to and above 10 units daily.

The results of Bülbring (15), if analyzed separately with respect to diet and dosage of parathormone, are in accord with our own. Although, in determining the calcium and phosphorus percentages of the dry extracted bones, she used the average of the entire group as indicating the parathormone effects, analyses of the bones of each in-

dividual animal show that the differences in the percentages of lime were due to the variability in the doses of parathormone employed. Thus on the stock diet (minimal calcium and phosphorus), used by her, the calcium percentages varied between 15.4 and 21.5; and on the high calcium diet between 13.2 and 20.9, the variation being due not to extraneous factors but to the amount of parathormone injected. Since it was pointed out previously that, unless there is a preponderance of osteoid or fibrous tissue over calcified tissue, the percentages of lime in the dry extracted bones of parathormone-treated animals do not differ much from that of normal bones, reduction in the calcium percentages in Bülbring's animals and in ours occurred only when the dosage of parathormone was excessively high and fibrosis was demonstrated histologically.

No definite explanation can be offered for the seemingly contradictory or reversal effects of parathormone with variations in dosage, *i.e.*, *hyperostosis* with dosages up to 10 units and *osteitis* or *hypostosis* with 20 units or more daily. Such variability is encountered, not infrequently, in the behavior of some drugs and extracts of the secretions of other glands. Thus, the physiologic effects of large doses of adrenalin, insulin, and viosterol may differ materially from, or be entirely opposite to, those obtained with small amounts, although it must be remembered that even the small doses of parathormone used in the rat experiments are, in reality, enormous doses for dog and man. For this reason alone, the results obtained on rats cannot, as yet, be transferred *in toto* to osseous derangement observed clinically, in an attempt to explain osteitis fibrosa as being due to slight or moderate hyperfunction and "marble-bone" disease as resulting from marked hyperfunction of the parathyroids (13, 23), although the finding of parathyroid hyperplasia in a case of "marble-bones" by Pehu, Policard, and Daufourt (32) does suggest such a relationship. The fact that osteitis fibrosa can be produced in the rat by a dose of parathormone larger than that which caused hyperostosis, namely, 20 units daily, does not speak for such a definite mechanism and indicates that, in the absence of methods of measuring parathyroid hyperfunction, the application of results obtained with the rat to clinical manifestations must be left, for the present, in the domain of speculation.

The relation of viosterol to the parathyroids will be discussed more

fully in a separate communication and hence will be mentioned here very briefly. From the results obtained with parathormone it is evident that the effects of parathormone and viosterol are not necessarily the same, or even complementary, as claimed by Taylor and his associ-

FIG. 9. Section of the tibia of a rat fed 10,000-D viosterol for twenty-six days. It is seen that there is dissolution of the matrix and nearly the entire disappearance of the osseous trabeculae but without replacement of the decalcified areas or the marrow cavity by fibrous tissue. Compare these osseous changes with the decalcification and fibrous replacement, induced by large doses of parathormone, shown in figures 3 and 7.

ates, but rather that they might be antagonistic. The differences between viosterol and parathormone are reflected not only in their effects on the levels of serum calcium and inorganic phosphorus but also in the histology and chemical composition of the bones. Nontoxic amounts of parathormone stimulate cartilage proliferation and osteoblastic activity but depress osteoclastic function, so that new trabeculae are forming and the old ones are not resorbed. The lime salt deposition occurring in the trabeculae does not exceed the formation of organic matrix and hence the ratios of ash and of calcium and phosphorus to organic matter in the bones are not increased, but actually somewhat diminished (fig. 8). On the other hand, vitamin D inhibits cartilaginous and osteoblastic proliferation but promotes osteoclastic activity so that its administration prevents or abolishes the cartilage overgrowth in rickets and causes the invasion of osteoclasts in the trabeculae. The increased lime salt deposition which follows vitamin D occurs at the expense of the organic matrix and the ratio of ash to organic matter (A/R) thus increases.

The effects of toxic doses of parathormone and of vitamin D on the bones are also dissimilar. Parathormone produces decalcification, but the decalcified areas and the adjacent marrow spaces are usually replaced by fibrosis tissue; whereas large doses of viosterol produce dissolution of the trabeculae without replacement of the resorbed areas with supportive tissue and without materially affecting the marrow cells. This contrast is illustrated in figures 3 and 7, showing decalcification by parathormone, and in figure 9, illustrating the toxic effects of large doses of viosterol, previously described by Shelling and Asher (17).

SUMMARY

1. The effects of parathormone on the bones of young rats vary with with the dose of the hormone and the calcium and phosphorus content of the diet:

(a) On the stock diet increasing amounts of parathormone, up to 10 units daily for a 50–60 gram rat, produce hyperostosis; while 20 units induce osteodystrophia fibrosa.

(b) On rachitogenic diets, parathormone fails to prevent rickets but stimulates osteoblastic activity and trabecular proliferation.

(c) On "border-line" rachitogenic diets parathormone stimulates cartilaginous proliferation as well as increased trabeculation.

(d) On optimal calcium-high phosphorus diets fibrosis occurs with a relatively lower dose of parathormone than on the stock diet.

2. Parathormone injections in the rat evoke moderate hypercalcemia and hyperphosphotemia; the hyperphosphotemia, however, depends on the dose of the hormone and on the phosphorus intake.

(a) On the stock diet, larger doses of parathormone increase the serum inorganic phosphorus nearly 100 per cent, but on the rachitogenic diet the serum inorganic phosphorus remains at rachitic levels.

(b) In one group of animals fed a low calcium-low phosphorus diet and injected with 10 units of parathormone daily, the serum calcium was 5.3 and the inorganic phosphorus 14.3 milligrams per cent. In spite of the hypocalcemia and hyperphosphotemia, there was no clinical evidence of tetany.

3. Analyses of percentages of ash and of calcium and phosphorus of the dry extracted femurs indicate certain dissimilarities between the osseous hypercalcification due to feeding vitamin D and that induced by parathormone. The former increases lime salt deposition in the *existing* organic matrix so that the ratio of ash to organic matter is increased, whereas parathormone first stimulates trabeculation, and the calcification that follows does not exceed the production of organic matrix, and hence the ratio of ash to organic residue is not increased but may be actually diminished if fibrosis and osteoid tissue are also present.

4. The injection of large doses of parathormone into young suckling rats produces cartilage proliferation, delayed ossification of the epiphyseal centers, decalcification and fibrous replacement of the metaphyses, but *not* typical rickets.

5. Large doses of parathormone produced, in ten-day-old suckling rats, lesions of the skin resembling scleroderma. These lesions, however, were absent in older rats so treated.

6. Metastatic calcification occurred only when the doses of parathormone were very high and when the bones were markedly decalcified.

7. Certain discrepancies in the literature as to the effect of parathormone in the rat are discussed and the divergent results explained as possibly due to variations in: (1) age of the animals, (2) dietary calcium and phosphorus, and (3) dosage of parathormone.

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PNEUMONECTOMY

A Preliminary Report of the Operative Technique in Two Successful Cases

By WILLIAM FRANCIS RIENHOFF, JR.

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The operative procedure described in this report has been successfully employed in two cases in which complete removal of the entire left lung was found to be necessary because of a benign and a malignant tumor, respectively, of the left primary bronchus.

For two weeks previous to operation, the left lung was compressed by a gradually induced artificial pneumothorax that finally brought about a complete collapse of that lung. The patients thus became accustomed to breathing with the non-collapsed lung and were thereby afforded an opportunity, before operation, of establishing their respiratory and circulatory equilibrium under the altered conditions of intrathoracic pressure which would prevail during and after the operation. The lung, when collapsed, is much more easily manipulated and requires far less handling for its removal than one that remains distended, and, besides, the exposure is vastly improved so that one can dissect the anatomical structures of the hilus without difficulty. The operation can therefore be performed more swiftly and with a minimal amount of shock from constant handling of and pulling on the structures of the hilus. Shock caused by the opening of the pleural cavity is avoided because the increased intrathoracic pressure, due to the artificial pneumothorax, is practically in equilibrium with the atmospheric pressure.

Avertin, 70 mgm. per kilo, was given by rectum thirty minutes before operation. Nitrous oxide and oxygen as a supplementary anesthetic was administered by Miss Olive Berger. Intratracheal anesthesia was not used. The patient, having become accustomed to breathing with one lung, experienced no difficulty when the chest was opened. This obviates the necessity and nuisance of inserting a

tube in the trachea, and also eliminates an otherwise unavoidable introduction of infection into the trachea and bronchi.

The position of the patient on the operating table was semi-recumbent, with the right side down. The trunk was rotated towards the right, to an angle of about 45°; the left arm was raised over the head, and the head of the table elevated to 45°. This position gave the best exposure of the antero-lateral surface of the left chest, throwing little if any weight upon the right side, and at the same time relieving the diaphragm of the weight of the underlying viscera.

After the usual iodine technique to the skin and draping of the operative field, an incision was made in the third interspace, parallel to the third and fourth ribs and extending from the costal cartilages to the anterior axillary line. The fibres of the pectoralis major were divided along their course. The internal intercostal muscles were cut directly across. The parietal pleura herniated up between the ribs as a result of the increased intrathoracic pressure, and when incised the escaping air gave off a hissing sound. The third and fourth ribs were merely spread apart with a self-retaining retractor. The anterior approach was used, as this gives a far better exposure of the hilus. The pulmonary artery and veins lie in front of the left primary bronchus and ligation of these vessels individually can be safely and easily accomplished when one encounters them anteriorly. No difficulty was experienced in ligating the vessels in either of these cases. The pulmonary artery running in front, and rather on the top of the left bronchus, was isolated by blunt dissection and clamped just midway between the reflection of the pericardium and the primary division of the artery. The proximal arterial stump was then ligated with oiled braided silk, and the mouth of the vessel transfixed with medium waxed silk. The superior and inferior pulmonary veins were then isolated in turn and transfixed with medium waxed silk. In the first case, operated upon July 24th, the left primary bronchus was cut across with the scalpel and the lung removed from the chest. The cartilaginous rings of the bronchial stump were cut at various points in their circumference in order to do away with their spring-like action, which normally tends to maintain the patency of the bronchus. The bronchus was sutured with interrupted medium silk sutures, the mucous membrane being approximated just as one would do in the

suture of mucous membrane elsewhere. In the second case, operated on November 3rd, the technique was as described, but owing to the location of the tumor it was possible to cut across the first ventral or superior branch and then incise the primary bronchus diagonally. This left to be closed two openings somewhat smaller than if the primary bronchus had been cut across perpendicularly; and the smaller the opening, the easier and more secure will be the closure. There was very little loss of blood during the course of the operation. The pulse rate and blood pressure were unaltered. The operations caused practically no shock to the patients and were not prolonged, the first one requiring thirty minutes and the second one and one-half hours. The ribs, which had been spread apart, were reapproximated with no. 2 silver wire and the pectoral muscles, subcutaneous tissue and skin were sutured with interrupted fine silk sutures. In both cases the chest was closed without drainage. The first patient, operated upon on July 24th, had an afebrile and perfectly uneventful convalescence. The anterior mediastinum has been gradually pushed over to the left side of the chest because of compensatory dilatation of the remaining right lung, which after 3 and one-half months has passed over the midline into, and practically filling, the left chest. This patient is a child of three and a half years and, in the course of three months, the remaining right lung has filled the entire space previously occupied by the left lung. The second patient, operated upon November 3rd, has also had an uneventful convalescence with *per primam* healing throughout. The successive roentgenograms of her chest since operation show that her heart has been displaced toward the left even at the early post-operative date of just over four weeks. Her right lung has already almost completely filled her entire chest, as occurred in the first case. In these cases the right lung crossed the midline anterior to the heart, thus entering the left thoracic cavity. The second patient is twenty-four years of age. The bronchus in both of these cases healed *per primam* with no bronchial fistulae or infection of the thoracic cavity resulting. Three successive post-operative aspirations of the chest, in both cases, revealed a very small amount of sero-sanguineous fluid which on bacteriological examination proved to be sterile.

It is not necessary to perform a thoracoplasty at the same time as

a pneumonectomy because the remaining lung will, in uninfected, clean cases obliterate the ensuing dead space. To prevent infection, mass ligatures and cauterization of the bronchus should be abandoned. Individual ligation of the vessels and the minimal amount of injury to the bronchus in suturing permits primary healing of the bronchial mucous membranes.

BOOK REVIEWS

Amerika und die Medizin. von Dr. Med. Henry E. Sigerist. Mit 30 Abbildungen und 2 Karten. Pp. 232. (*Georg Thieme Verlag, Leipzig. 1933.*)

Dr. Sigerist's book on American Medicine will prove as welcome in America as it is sure to be valuable in Europe. We Americans do not realize how little our European colleagues know about us; what imperfect ideas they have about our geography and especially about our medical life and practice. However, now that Dr. Sigerist's book has been published, there will be no excuse for a European physician if he still believes that San Francisco is just around the corner from New York and that medical education in America is so imperfect that our only good physicians are those who have studied abroad. But the book, especially when it has been translated into English, will be invaluable to Americans themselves. As a matter of fact, we possess no satisfactory study of the development of medical life and teaching in the United States. Any American reading Dr. Sigerist's book will learn a great deal that he never knew before, especially about the great pioneer personalities in American medicine and the history of medical teaching in our country. The translation of this book into English therefore will be eagerly awaited.

Nevertheless, I should advise any one who reads German easily to read Dr. Sigerist in the original. He possesses a very unusual style, that is brilliant, balanced and vivid. His introduction (pages 7–11) and his First Chapter (Der Boden, pages 15–28) are good examples of how in a few pages he can paint a brilliant historical picture against a tremendous background and yet with fascinating details. Some of his chapters remind one of some of the great frescoes in Italy, especially those of Signorelli at Orvieto. Dr. Sigerist is especially fortunate in his biographical work. In Chapter IV, on "Pioneers," he gives the biographies of fifteen or twenty great American physicians, beginning with John Morgan and Benjamin Rush and ending with Sir William Osler. Those who have already read Dr. Sigerist's "Grosse Aerzte" published last year and now translated into English will enjoy these biographies especially.

To everyone interested in medical education, I commend Chapter V (pages 151–198). Most of us do not realize, for example, how many American medical students have been going to Europe since the war for their medical education. Before the war, an American who took all his medical work in Europe was a very rare bird. Since 1920, many men, who want to study medicine, have been unable to secure admission to our own medical schools and have turned to Europe. These men, therefore, who now go to Europe represent the lowest level of our medical aspirants, since they have not been able to fulfil the requirements of our own in-

stitutions. Dr. Sigerist shows that many of these men who now take their medical degrees abroad fail lamentably to pass the examinations of the State Boards after their return home. In the old days we sent to Europe our best men as post-graduate students. Now we are sending our worst. Dr. Sigerist's figures can not help causing us some anxiety about the future of our profession. We do not realize that there are at present so many young Americans anxious to study medicine that our own medical schools, in spite of their extent and number, can not accept them all and must leave to the European schools those applicants who have been rejected at home. This is only one example among many that may be cited to show how Dr. Sigerist's book can often tell us more about ourselves than we ourselves know or even suspect.

To be able to write a book of this kind after twenty years in America would have been a remarkable achievement. To have written it after three or four years' knowledge of American conditions is so unusual that one seeks in vain for a parallel in all medical literature. Finally, when one realizes that the book was written during such hours as could be spared from days that were devoted to frequent lectures, much teaching and constant personal contacts and conferences with the writer's own staff, one not only appreciates the book, but one admires also the man who wrote it.

The book ends with a glance towards the future. Dr. Sigerist believes that American medicine reaches the peak of its modern achievements in Dr. Welch's work and in his founding of the Johns Hopkins Institute of Medical History. Dr. Sigerist looks forward now to a study of the medicine of Russia, for he feels that Russian and American medicine may reach new developments and new adjustments that would never be possible in other lands.

The book is dedicated to Col., Fielding H. Garrison, the pioneer of medical history in America, and is delightfully illustrated.

J. R. O.

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